HISTOPATHOLOGICAL EVALUATION OF RENAL BIOPSIES IN CASES OF NEPHROTIC SYNDROME WITH SPECIAL REFERENCE TO USE OF IMMUNOFLUORESCENT STAINING IN SELECTED CASES

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CHENNAI-TAMILNADU

DEDICATED TO MY PARENTS

MY HUSBAND AND SON.

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"Nothing gives a better perspective of the subject than an appreciation of the steps by which it has reached its present state" – Dr. Conrad L. Pirani.

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CERTIFICATE

This is to certify that the dissertation entitled " HISTOPATHOLOGICAL EVALUATION OF RENAL BIOPSIES IN CASES OF NEPHROTIC SYNDROME WITH SPECIAL REFERENCE TO USE OF IMMUNOFLUORESCENT STAINING IN SELECTED CASES" Presented herewith by Dr. R. SANTHI to the faculty of Pathology. The Tamilnadu Dr. M.G.R. Medical University, Chennai in Partial fulfillment of the requirement form the award of M.D. degree in Pathology is a bonafide work carried out by her under my direct supervision and guidance.

CONTENTS

INTRODUCTION

INTRODUCTION

The diseases of the kidney are fascinating which can have varied of clinical presentations, with no two patients being alike.

In our country, Renal diseases form an important cause for morbidity and mortality. Nephrotic syndrome is an important and common clinical presentation. The causes of nephrotic syndrome are numerous, which can be either primary or secondary. Once the secondary forms of nephrotic syndrome are excluded by appropriate clinical and laboratory data, there remains a group of patient with idiopathic nephrotic syndrome who can be precisely differentiated only by renal biopsy. In children with idiopathic nephrotic syndrome, empirical steroid therapy is the initial treatment of choice. However in children who fail to respond to a course of steroids, renal biopsy may be indicated, to provide a specific diagnosis.

The primary role of renal biopsy is to provide a definite diagnosis that allows the clinician to assign lesion – specific therapy.

There has been tremendous growth in the field of immunology, especially pertaining to nephrology, which is useful in the clinical diagnosis of the condition, helpful to prognosticate and predict therapeutic response.

The present study has been taken–up with an earnest attempt to find out the common histopathological patterns of renal diseases which clinically present with features of nephrotic syndrome.

AIM OF THE STUDY

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1. To evaluate the histopathological lesions found in the kidneys of nephrotic patients.
2. To estimate the statistical occurrence of different types of Glomerulonephritis which clinically present with nephrotic manifestations.
3. To evaluate the clinicopathologlical correlation of different types of glomerulonephritis whenever possible.
4. To correlate the pathological findings with immunofluorescence technique studied in selected cases.
5. Comparative evaluation of data of the present study with those from other study centers.

REVIEW OF LITERATURE

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ANATOMY

The kidneys are situated retroperitoneal in the posterior part of the abdomen on either side of the vertebral column and surrounded by layers of fat, fascia and areolar tissue. The upper poles of the kidney are approximately at the level of the 12th thoracic vertebra. Their lower poles are at the 3rd lumbar vertebra.

The right kidney is slightly lower than the left, a circumstance usually ascribed to the limiting position of the liver. The long axis of each kidney is directed downward and laterally and the transverse axis posterior and laterally.

Each kidney measures about 11 x 6 x 2.5cm, the left being somewhat longer and narrower than the right. The kidneys of the adult men weigh from 125 to 170 gms each and those of the adult women weigh 115 to 155 gms each.

Development

The definitive human kidney arises from two distinct sources. The excretory tubules (or nephrons) are derived from the lowest part of the nephrogenic cord. This part is the metanephros, the cells of which form the metanephric blastema.

The collecting part of kidney is derived from a diverticulum called ureteric bud which arises from the lower part of the mesonephric duct.

Vertebrate kidney has passed through three stages of evolution. The most primitive of these is called the pronephros. It is the functioning kidney in some cyclostomes and fishes. This has been succeeded in higher vertebrates by the mesonephros, which is the functioning kidney of most anamniotes. The kidney of amniotes **(**including man) is called the metanephros.

Histology

The kidney is subdivided into an outer region the cortex and an inner region the medulla. Externally the cortex is covered with a connective tissue capsule and the perirenal connective tissue.

In the cortex glomeruli, convoluted tubules, straight tubules and medullary rays are found. The medulla comprises a number of renal pyramids.The apices of renal pyramids form the papilla which projects into a minor calyx. The medulla also contains the loops of Henle and collecting tubules.

From the standpoint of kidney diseases , the kidney can be divided into four compartments ; glomeruli, tubules, blood vessels and interstitium. The kidney is richly supplied by blood vessels, receiving 25% of cardiac output.

Blood vessels

The main renal arteries at the hilum are divided into anterior and posterior sections. From these interlobar arteries arise and give rise to arcuate arteries which arch cortex and medulla and inturn giving rise to interlobular arteries .

From the interlobular arteries, afferent arterioles enter the glomerular tuft where they progressively divide into 20 to 40 capillary loops arranged in several lobules . Capillary loops merge together to exit from the glomerulus as efferent arterioles.

Glomeruli

The glomeruli consist of anastamosing network of capillaries lined by fenestrated endothelium invested by two layers of epithelium. The visceral epithelium is incorporated into and becomes an intrinsic part of the capillary wall separated from endothelial cells by a basement membrane.

The parietal epithelium lines bowman space, the cavity in which plasma filtrate first collects.

The glomerular capillary wall is the filtering membrane and consists of the following structures.

- A thin layer of fenestrated endothelial cells, each fenestrum being about 70 to 100 nm in diameter
- A glomerular basement membrane(GBM) with a thick electron-dense central layer, the lamina densa and thinner electron-lucent peripheral layers, the lamina rara interna and lamina rara externa.
- The visceral epithelial cells(podocytes), structurally complex cells that possess interdigitating processes embedded in and adherent to the lamina rara externa of the basement membrane.
- The entire glomerular tuft is supported by mesangial cells lying between the capillaries. Basement membrane-like mesangial matrix forms a meshwork through which the mesangial cells are scattered.

Tubules

The structure of renal tubular epithelial cells varies considerably at different levels of the nephron.

Numerous tubules lie adjacent to the renal capsule. The tubules are primarily of two types, the proximal convoluted and distal convoluted. The PCT contain a small uneven lumen with single layer of large cuboidal cells with intense eosinophilic, granular cytoplasam and well developed brush border. The DCT are fewer in number and exhibit a larger lumen with smaller cuboidal cells and the brush border is not present.

Interstitium

In the normal cortex, the interstitial space is compact, being occupied by the fenestrated peritubular capillaries and a small number of fibroblast-like cells.

RENAL BIOPSY: AN HISTORICAL PERSPECTIVE

Until about 1950, what was known about the pathology of renal diseases was based almost exclusively on post-mortem studies.

Gwyn [45] (1923) was the first to suggest that when indicated, a renal biopsy should be taken at the end of abdominal surgeries.

The possibility that a percutaneous needle biopsy could be obtained from a kidney was first demonstrated by Ball RP[8] (1934) who biopsied only palpable tumours.

Lindblom [74] (1946) used a needle and by injecting diodrast was able to differentiate renal cysts from solid tumours. The percutaneous renal biopsy was introduced by Iverson and Brun[59] (1951) in Denmark.

The first to use needle for the diagnosis of medical diseases of the kidneys were Alwall [3] in Sweden (1952) – aspiration biopsy of kidney and diagnosed as amyloidosis.

Kark [65] (1954) introduced several important changes in Iverson and Brun technique.

Today, practically all biopsies taken with one of the many different methods to visualise the kidney and the position of needle.

Technical Advances

There have been numerous attempts to classify glomerulonephritis over the years and notable among them are those of Volhard and Fahr[93] (1914) Addis[1] (1931) and Ellis.A[36] (1942). All were devised before the renal biopsy was introduced and all were dependent on autopsy material for the pathologic aspects when clinicopathologic correlations were made. The percutaneous renal biopsy was introduced as a relative safe routine procedure by Iversan and Brun [59] (1951). Alwall.N [3] (1952) had used a technique of aspiration biopsy of kidney and diagnosed amyloidosis.

The availability of fresh tissue allowed electron microscopic studies to be carried out so that over the year the combined use of light, electron and immunohistologic miscroscopic studies on renal biopsies coupled with appropriate experimental models led to our present concepts and classification of renal disease.

For Light microscopy, in addition to hematoxylin-eosin the trichrome stain of Masson and the periodic acid Schiff reaction war used first by Mc Manus.JFK[77] in 1948. Most importantly Jones[62] (1951) and then Churg and Grishman[26] (1957) emphasized the importance of thinner section (2-3 μm) particularly for the visual resolution of glomerular lesions.

Jones D.B[64] (1953) introduced the methanamine silver stain which gave superior results for basement membrane.

Hall's colloidal Iron stain for the mucosubstances of the glomerulus was introduced some what later by JonesD.B.[63] (1969)

Electron microscopic studies

For E.M Studies, at first using osmium tetraoxide both as a fixative and as a staining solution the minute tissue fragments were embedded in Methylmethacrylate by Cosselett.V.E [29] (1951).

Glutaraldehyde fixation was followed by post fixation in osmium tetraoxide embedding in Epon or other plastic material [84]. Diamond knives were used for sectioning[37].

Immunopathology

The first symptomatic study on renal biopsies was published by Castleman and Smithwicke[21] in 1943 as 'Renal Biopsy" . Von Pirquet [94] (1911) first described the importance of immunology when he noticed that there was delay between administration of a foreign serum and development of sickness and postulated that host antibody combined with the injected serum to form 'A Toxic Compound' which we now realize to be immune complex deposited in the kidney .

Krakower C.A and Greenspan S.A [69] (1951) had demonstrated that the nephrotoxic antigen responsible for Masugi nephritis was localised in the glomerular capillary basement membrane.

Dixon et al[35] in (1961) worked on renal biopsies and showed that deposition of immune complexes in the glomeruli coincided with development of proteinuria and hypocomplimentemia. These deposits were detectable by light microscope and by electron microscope.

Stokes first used the word 'Fluorescence' to describe a reaction of Fluorespur to ultra violet light. The application of the filter which absorbed visible rays and allowed only U.V. light to be transmitted lead to the discovery of the first fluorescence microscope. Subsequently Max Haitinger developed the technique of staining histological preparation with fluorescence dyes. The same technique is used even today.

The fluorescent antibody labelling technique of Coons and Kaplan[28] reported in 1950 permitted for the first time, the identification of antigens and antibodies. They used fluorescein isocynate which imparts green fluorescent colour to the tagged antibody that is distinguished from tissue autofluorescence. Isocyanate is now replaced by fluorescein isothiocynate (FITC) over the last many years. This is more stable, cheaper and gives more intense green fluorescence.

An alternative method for immunofluorescence is – immuno peroxidase staining technique. This technique depends on the localization of antigens in tissues using labeled and unlabeled antibody sequences or enzyme antibody conjugates coupled with chromogenic histochemical reactions. This technique is widely used in all tissues including renal biopsy.

NEPHROTIC SYNDROME

The loss of protein in urine is one of the principal clinical manifestations of renal disease and when it exceeds 1.0 gm/24 hrs it usually signifies glomerular involvement.

Ellis[36] (1942) separated glomerulonephritis (GN) on the basis of clinical and laboratory findings into Two types – Nephritic glomerulonephritis (Ellis type I) and Nephrotic glomerulonephritis (Ellis type II).

Nephrotic GN is delineated by an absence of any preceding history of bacterial infection, normal anti streptolysin titres, insidious onset of marked edema, albuminuria without significant haematuria, ascites, hyperlipidemia, normal blood pressure, absence of azotemia and a poor prognosis.

Jones[63] (1969) studied the renal biopsies with the help of periodic acid methanamine silver (PAMS) stain and observed that on clinical and pathologic grounds, nephrotic GN and nephritic GN have similarities and differences. Pathologically the types of glomerular lesion seen in nephritic GN have been divided into minimal lesion of childhood, the moderate lesion of the uraemic child or young adult, the chronic lobular lesions and the membranous lesions.

White et al[96] (1970) studied the renal biopsy specimen from 145 children with the nephrotic syndrome and placed these in four morphological categories: Minimal change disease (MCD), focal segmental glomerulosclerosis (FSGS), proliferative glomerulonephritis and membranous glomerulo nephritis (MGN).

Hayslett et al [49] (1973) studied over a 15 years period 95 patients with the nephrotic syndrome and revealed that the course of the nephrotic syndrome and its response to specific forms of therapy are closely related to the type and severity of the renal lesion. Corticosteroid therapy is useful in reducing protein excretion only when used to treat patients with lipoid nephrosis and mild proliferative GN.

Bohrer M.P[15] et al (1978) studied the normal glomerular capillary wall which filters serum protein from the ultra filtrate on the basis of both the size and the change of the macromolecules and both of this discriminatory function may be impaired in nephrotic sydrome.

Kaysen . G.A and Schovn feld Y[66] (1984) found that the tubular catabolism of filtered and reabsorbed albumin contributes to the total protein loss but the inability of liver to appropriately increase protein synthesis in response to protein loss, also plays a role.

According to Le J, Vilcek.J[73] (1989) depressed albumin synthesis may be explained by cytokines which depress hepatic protein synthesis when released during glomerular inflammation.

Glassock RJ[44] (1995) identifies more than 90 causes for the nephrotic syndrome and 90% of the cases of nephrotic syndrome in children and 70% in adults are due to three patterns of glomerular injury-minimal change glomerulopathy, focal segmental gromerulo sclerosis and Membranous nephropathy and each of those pattern occurs in primary or idiopathic form and secondary forms with known causes and association.

Haas M et al [48] (1997) reviewed data from 1,000 adult native kidney biopsies performed in cases with a full-blown nephrotic syndrome between two time intervals and compared the relative frequencies of specific diseases. They concluded that the commonest cause of nephrotic syndrome in earlier study was changed from MGN to FSGS in later study.

Bazzi et al[10] (2000) evaluated 89 patients with nephrotic syndrome to determine if the selectivity of proteinuria was associated with tubulointerstitial damage... When proteinuria is highly selective, the tubulointerstitial damage is rather infrequent, and 100 % of patients develop clinical remission. When proteinuria is moderately selective or non-selective, increasing number of patients develop tubulointerstitial damage.

According to Vande Walle JG et al [92] (2001) based on observations in 110 children with nephrotic syndrome, the basic abnormality is a primary disturbances in renal sodium excretion.

According to Chandrasekaran et al [22] (2001) the commonest cause of nephrotic syndrome was Diffuse proliferative glomerulonephritis (DPGN).

Chaturvedi M et al[23] (2001) encountered the glomerular histopathology in patients of adult nephrotic syndrome which is different from those seen in childhood nephrotic syndrome. They evaluated the histopathology in 200 adult patients who presented with nephrotic syndrome. Of these 200 patients, 160 [80%] patients had idiopathic nephrotic syndrome while 40 (20 %) patients had secondary nephrotic syndrome.

The predominant histopathological lesions in patients with idiopathic nephrotic syndrome were:Membranous Glomerulonephritis (MGN) followed by Membranoproliferative Glomerulonephrirtis (MPGN} and focal segmental glomerulosclerosis (FSGS}. Amyloidosis followed by Lupus Nephritis was the major secondary causes.

Among the patients who presented with deranged renal functions the commonest lesion was MPGN followed by MGN and FSGS and in patients with microscopic hematuria, the commonest histopathology was MPGN followed by MSPGN and Figs. patients presented with hypertension, the commonest lesion was MPGN followed by FSGS.

S F Haque and A S Khan[47] (2001) studied the clinical features and clinico-pathological correlation in adult patients with nephrotic Syndrome. MPGN was the commonest followed by MSPGN, MGN, DPGN, FSGS and Amyloidosis. Highest number of Steroid-non-responders where in MSPGN (70 %) followed by MGN(48%). In this brief series of adult-onset nephrotic syndrome, Membrano proliferative glomerulonephritis was found to be the commonest type.

Balakrishnan N et al [6] (2001) conducted retrospective analysis of the histopathology reports of native kidney biopsies performed and found that most of the biopsy proven renal diseases (BPRD) showed male predominance except in Lupus and cortical necrosis. BPRD in children showed highest incidence of MCD followed by FSGS and mesangioproliferative GN.

This study substantiates FSGS as the commonest cause of adult nephrotic syndrome as observed in recent western studies.

Minimal change glomerulopathy

The term Nephorsis was first used by Muller in 1905 and the term lipoid nephrosis was coined by Munk in 1913 to describe a group of patients with oedema, hypoproteinemia and hyper cholestroelemia. Later Volhard and Fahr[93] (1914) used this term to denote a renal condition in which an inflammatory component was absent.

White et al[96] (1970) studied the renal biopsy specimens from 145 children with nephrotic syndrome. According to them, the commonest one is minimal change glomerulopathy and children with MCD who were mainly boys (M/F ratio 1.6:1) and of preschool age, generally had highly selective proteinuria, while haematuria was present in 13 % and hypertension in 9 % of patients.

Churg J et al[25] (1970) studied renal biopsies from 127 children with the nephrotic syndrome and observed minimal changes in 77 % of patients .According to them, the group of patients showing MCG on biopsy is distinguishable from the other groups by features in addition to the histological findings. These features include a younger age distribution, a preponderance of affected boys and infrequent occurrence of hypertension and haematuria, which are transient when they do occur.

Korbet et al [67] (1988) studied that when the patient is under 2 years of age it account for 90% of cases**.**

It also occurs in adults in whom it accounts for a smaller percentage of cases of nephrotic syndrome. According to them, the mean age was 40 in adults and females were commonly affected.

Postishil [82] (1995) found that it is most often primary disease but some identical conditions were caused by NSAIDS.

According to Sibley RK [88] et al (1994) frequent association of minimal change with atrophy and it occurrence with lympho reticular malignancies.

Histological variants

On histological diagnosis of nephrotic syndrome in 1,810 children MCG accounts for 66% and in 985 adults it accounts for 22%.

The international study of kidney disease in children (ISKDC) evaluated five variants of MCG including 98 FSGS, 16 cases of mild mesangial thickening **(**increased PAS positive matrix), 29 cases of focal tubular changes, 27 cases of mild mesangial hypercellularity and 12 cases of diffuse mesangial hypercellularity

Other significant variants of MCG are identified by diffuse mesangial sclerosis (IgM Nephropathy) [58].

Secondary Minimal Change Disease.

Postishil[82] (1995) studied a similar or identical lesion that can appear consequent to a growing number of underlying diseases; it is then known as 'secondary minimal change disease'. The predisposing conditions include neoplastic diseases, toxic or allergic reactions to drugs, infections, auto-immune disorders and other miscellaneous entities.

Focal Segmental Glomerulo Sclerosis

FSGS was first described in an autopsy study of children and young adults who presented with nephrotic syndrome and developed a progressive renal failure and hypertension .

Cheigh JS et al[24] (1983) reported frequent recurrence of FSGS after transplantation. FSGS is found in 7% to 15% of children and 15% 20% of adults It is more common in males. Ellis et al[36] (1978) suggested that the presence of vascular changes is indicative of a poor prognosis and a tendency to develop hypertension. Cameron[20] (1978) suggested that the children in whom FSGS is documented in an initial renal biopsy have worse prognosis.

Beaufils et al [11] (1978) studied renal biopsies from 70 patients by light microscopy and revealed that all the patients showed focal and segmental involvement of glomeruli. The glomerular lesion was characterized by the presence of segmental acellular glomerulosclerosis. The sclerotic segments contained eosinophilic deposits with frequent lipid like vacuoles and often foam cells. The involved capillary loops were often directly adherent to Bowman's capsule and epithelial crescents were rare.

According to Howie AJ et al [57] (1993), in light microscopy segmental sclerosis usually affects one or more lobules of the glomorular tuft near the axial region, often appearing to adhere to Bowman capsule. Early lesions show increased mesangial matrix and hypercellularity. Only when the sclerosis advanced the area become hypocellular.

Hass M et al [48] (1995) and Srivastava T et al (1999) reported an increase in the incidence of FSGS in adults and children .

Shiki H et al [87] (2000) found that the primary FSGS is the representative of refractory nephrotic syndrome in both adults and children and they frequently develop end stage renal disease whereas patients achieving remisson show an excellent outcome.

According to Howie AJ[55] (2003) sclerosis is seen in the portion of the glomerulus opposite the hilus forming an adhesion in the vicinity of the opening of the Bowman space into the proximal tubule (Glomerular tip lesion)

C1q nephropathy: a variant of focal segmental glomerulosclerosis:-

C1q nephropathy is a poorly understood and controversial entity with distinctive immunopathologic features. Defining criteria included (1) dominant or co-dominant immunofluorescence staining for C1q, (2) mesangial electron dense deposits, and (3) no clinical or serologic evidence of systemic lupus erythematosus (SLE).

C1q nephropathy falls within the clinical-pathologic spectrum of MCD/FSGS. Markowitz GS.et al [76] (2003) hypothesized that it may be a non-specific marker of increased mesangial trafficking in the setting of glomerular proteinuria.

Pathology of minimal change nephropathy and segmental sclerosing glomerular disorders:-

Segmental sclerosing glomerular disorders are often called focal segmental glomerulosclerosis, one of the most controversial terms in kidney disease. The tubular opening is the earliest site at which segmental changes appear. These tip changes are not a disease in themselves. The glomerular tip lesion has tip changes in otherwise normal glomeruli. Tip changes in large glomeruli with mesangial increase can be called early classical segmental sclerosing disease. This can progress to give abnormalities at various sites, or late classical segmental sclerosing disease, corresponding with the classical descriptions of focal segmental glomerulosclerosis. Hilar abnormalities are a characteristic finding in reduced glomerular numbers. According to Howie AJ.[55] (2003) Focal segmental glomerulosclerosis is an ambiguous term, applied to many different types of segmental sclerosing glomerular disorders.

Wang S et al[95] (2004) studied that damaged podocytes may exhibit p27 and p57 protein expression in the cellular lesion of FSGS detetectable by immunohistochemistry and immunoelectron microscopy.

IgA Nephropathy

In 1968 Berger and Hinglais[13] described a disease entity primarily characterized by mesangial proliferative changes and diffuse Immunofluorescent mesangial deposits.

Clarkson AR, et a[27]l stated that it was more common in second or third decades and males were affected at a rate of three to six times more often than females. According to Galla JH [40] (1995), roughly 5% to 10% develop the nephrotic syndrome.

Histopathology

Hogg RJ[52] (1988) studied spectrum of clinical features observed in pediatric patients with IgA nephropathy.The typical clinical presentation consists of an episode of macroscopic hematuria within 24 to 48 hours of upper respiratory infection. Hypertension and proteinuria are observed frequently in patients who progress to chronic renal failure.

According to Daniel L [31] (2000) the tubular lesions determine the prognoisis of IgA nephropathy. Genel F et al [42] (2001) studied that IgAN charecterised by diffuse mesangial deposits of IgA detectable in immunofluorescence study. Decramer S [34] (2002) concluded that the most important prognostic indicaters of Ig A nephropathy are diffuse tubulointerstitial lesions and extracapillary proliferation with crescents , in more than 50% of the glomeruli. A correlative investigation between the clinicopathological features and outcome of idiopathic IgA nephropathy with diffuse crescent formation concluded that the patients of IgAN with diffuse crescent formation show rapidly progressive glomerulonephritis. [90] The glomerular epithelial cells (GEC) play an important role in glomerular filtration of the kidney. The disruption of these cells contributes to the development of glomerulosclerosis.

Membranous glomerulonephritis

Membranous glomerulonephritis (MGN) or epimembranous nephropathy is a histopathological pattern characterised by epimembranous and intramenbranous immune complex deposits and variable basement membrane thickening without mesangial proliferation.

According to Hayslett JP et al [49] (1973), it is a disease of adults with an age peak incidence of 40 years. It accounts for 20 to 30% of all cases of Idiopathic nephrotic syndrome(INS) in adults

According to Kuroki A et al [71] (2002) IgG4 was the predominant glomerular IgG subclass in MGN in immunofluorescence study. Passos EM et al [80] (2003) defined membranous glomerulonephritis as a chronic glomerular disease characterized by presence of subepithelial deposits along the glomerular basement membrane. They further studied the renal biopsies by immunofluorescence and found that these subepithelial deposits always fix anti immunoglobulin G (IgG) serum.

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Histologic staging of MGN

According to Ferrario F et al[38] (2004) sub epithelial deposits in MN undergo an orderly incorporation in to the GBM as a part of healing process and this is the basis for staging.

Stage	L.M	E.M
I	No change	Sub epithelial electron dense deposit
II	Spikes	Sub epithelial deposits with intervening BM like material
III	Complex Pattern twisted rope	Incorporation of deposits into the basal lamina
IV	Complex pattern	Reabsorption of deposits with loss of electron density
V	Return to Normal	Remodeling and normalisation of basal lamina

Congenital nephrotic syndrome(CNS)

The nephrotic syndrome is common in the first year of life. According to Habib R[46] (1993) in one series of 1000 Children with the nephrotic syndrome only thirty seven were under 1 year of age at the time of diagnosis. Sibley R.K and Striegel J[88] (1994) studied that renal biopsy is essential in order to differentiate the two types of CNS from other renal disorders of the neonatal period, including membranous nephropathy, congenital toxoplasmosis, HIV, Malaria, Cytomegalic inclusion disease and minimal change disease.

Finnish Type:

Seppala M and Rajpola J[86] (1976) suggested that the diagnosis may be established in utero from the characterstic family history and the findings of increased alpha fetoprotein levels in the amniotic fluid and maternal serum.

According to Huttunen N P[57] (1976) the patients have large placenta at birth, proteinuria, oedema and a high susceptibility to infections.

Habib R[46] (1993).found that Congenital NS of Finnish type makes up fewer than 1.5% of cases of NS in childhood.Holmberg C and Jalanko H[53] (1991) revealed that most patients die of complications of the NS by the age of 3 years, but those who servive and receive transplants usually show dramatic improvement in their psychomotor development and the nephrotic syndrome does not recur.

Garty BZ et al[41] (1994) found some evidence that glomerulopathy was inherited as an autosomal recessive trait.

MEMBRANOPROLIFERATIVE GLOMERULONEPHRITIS

Habib et al [46] (1961) and Mackay et al [75] (1961) studied the renal biopsies with the help of light microscopy and observed changes, including an increased glomerular cellularity, thickening of the BM of the glomerular capillaries, lobulation of the glomerular tuft and occasional formation of wire loops.

Jennings et al[61] (1961) revealed that these histopathologic changes are distinct from those noted in acute proliferative GN. They pointed out the rarity of true membraneous changes in proven patients of acute G.N. They also observed excess periodic acid Schiff (PAS) positive and fibrillar material in the regions of axial hypercellularity but diffuse membranous changes were not present.

Lawrence et al[72] (1963) stated that diffuse membranous changes in proliferative renal disease rules out the diagnosis of acute proliferative GN.

Burkholder et al[17] (1970) studied 16 renal biopsies from 11 patients with mixed membranous and proliferative GN by light microscopy (LM), Immunofluorescence (IF) and electron microscopy (EM). In their study by routine light microscopy, the major glomerular alteration consisted of mesangial hypercellularity and increase of BM material, thickening of peripheral capillary walls and lobular accentuation of the capillary tufts.

Fox[39] (1975) revealed the morphology in a deposit like lesion of glomerular and extraglomerular BM which can be identified and differentiated from other forms of MPGN with special thin section light microscopic techniques.

Burkholder[18] (1977) classified this lesion as membranoproliferative GN type II (MPGN II), although the abnormality is not limited to the glomerulus. They also revealed that although superficially similar, MPGN II is easily distinguished from mesangiocapillary GN (MPGN I and III). The glomerular subendothelial deposit lesions of MPGN I and III are contiguous mesangial subendothelial and there are no homologous extraglomerular abnormalities.

Davis et al[33] (1978) revealed that the consistent light microscopic feature of MPGN II is the presence of histochemically homologous deposits or deposit like alterations of renal glomerular and extraglomerular basement membranes.

According to Burkholder et al[17] (1970), examination of clinical and laboratory data suggests that mixed membranous and proliferative GN may be the result of possible progression of different renal diseases or of simultaneous or sequential occurrence of several hypersensitivity induced renal diseases. They correlated the ultrastructural and light microscopic findings and indicated that the PAS positive, masson-green thickening of the glomerular capillary walls is the result of thickened or expanded basement membrane containing electron dense deposits or displaying electron dense transformation. Enlargement of mesangial region in such patients is the result of an increase in endocapillary cells (mesangial and endothelial cells) and basement membrane like material, usually free of electron dense deposits.

Burkholder et al[17] (1970) found that immunofluorescence for localized immunoglobulins or complement did not always correlate with the size and distribution of these electron dense alteration, suggesting that not all of the electron dense deposits contain immunoglobulin.

Electron microscopic study by Burkholder et al [17] (1970) revealed that the membranous thickening of the glomerular capillary walls noted by LM was the result of thickened or expanded BM containing electron dense deposits, diffuse electron dense transformation or combination of subendothelial and subepithelial electron – dense deposits.

Fox[39] (1981) studied renal biopsy specimens from 16 patients with MPGN. A light microscopic study of MPGN identifies 3 morphologic patterns with homologous extraglomerular lesions. These correspond to reported variants of patterns of MPGN II (Dense deposit disease) by EM. They described endomembraneous, subendothelial and holomembranous patterns, all with homologous lesions of extraglomerular basement membranes. The holomembranous pattern is morphologically the most unique and may represent a different disease or a subset of MPGN II. The findings are consistent with the definition of MPGN I and III as mesangiocapillary GN and MPGN II as a nephropathic lesion not limited to the glomerular capillary basement membrane (MPGN with homologous extra glomerular lesions).

MESANGIO PROLIFERATIVE GLOMERULONEPHRITIS

Jennings and Earle [61] (1961) first described a pure mesangial proliferative glomerulonephritis during the resolution of post streptococcal glomerulonephritis.

Churg et al[25] (1970) and Glasgow et al [43] (1970) and studied and described a mild or pure mesangial proliferative glomerulonephritis in association with a variety of clinical syndromes, including idiopathic nephrotic syndrome, recurrent primary haematuria, Henoch – Schonlein nephritis and systemic lupus erythematosus.

According to Bhasin et al [14] (1978) mesangial injury glomerulonephritis is a glomerulopathy, characterized by mesangial IgM deposits and responsible for nephrotic syndrome.

Brown et al[16] (1979) in their study observed that a pure mesangial proliferative glomerulonephritis does not represent a specific disease entity but rather a non specific glomerular response to a variety of injuries.

Glasgow et al[43] (1970) studied and described recurrent episodes of haematuria in association with a mesangial proliferative renal lesions in children and adults.

Cameron et al [19] (1974) in their study reported a pure mesangial proliferative glomerular lesion in children and adults with idiopathic nephrotic syndrome.

Bhasin HK et al[14] (1978) studied and revealed that the most common manifestation is heavy proteinuria, often in nephrotic range, sometimes with microscopic haematuria and this lesion does not regularly respond to corticosteroid therapy.

Bhasin HK et al [14] (1978) studied renal biopsies by light microscopy and observed diffuse mesangial cell proliferation and matrix increase without involvement of peripheral capillary loops. In the early stages, mesangial widening and matrix and cell increase is generally mild but later there is sclerosis, sometimes segmental with hyaline foci and adhesion.

Bhasin HK [14] (1978) studied renal biopsies by immunofluorescence and found diffuse IgM deposition throughout all mesangial region and mesangial staining for Ig (or) complement.

Brown et al[16] (1979) studied the clinical course of the patients with mesangial proliferative glomerulonephritis who presented with haematuria and asymptomatic proteinuria suggested that this lesion had a tendency to resolve spontaneously.Brown et al [16] (1979) on light microscopy, observed a slight but diffuse increase of mesangial cells. The capillary loops were widely patient, and there was no evidence of epithelial proliferation or adhesions.

Baldwin et al [7] (1977) proposed a histological classification of lupus nephritis. They identified three fairly distinct forms with rather typical clinical features, focal proliferative, diffuse proliferative and membranous lupus nephritis. They observed transitions between the different forms and progression to glomerular sclerosis. They also observed necrotizing vasculitis and rapidly progressive renal failure in the course of diffuse proliferative lupus nephritis and a new pathologic form, mesangial lupus nephritis.

Baldwin et al[7] (1977) studied 88 patients with lupus nephritis and observed that focal proliferative lupus nephritis generally follows a benign course except in the occasional instances when transition to the diffuse proliferative or membranous forms occurs. Membranous lupus nephritis when characterized by persistent nephrotic syndrome, leads slowly to renal failure, but this progression is aborted in one third in whom remission of the nephrotic syndrome can be achieved. A fatal outcome occurs within 5 years in the majority of those with diffuse proliferate lupus nephritis and the nephrotic syndrome, often in association with necrotizing renal vasculits, severe hypertension and accelerated renal failure. They observed that mesangial immune deposits with or without proliferation, at times in the absence of clinical renal disease, early in the course of SLE may proceed to diffuse proliferate or membranous forms. Mesangial lupus nephritis was characterized by mild clinical evidence of renal disease, usually proteinuria and microhaematuria combined, occasionally by microhaematuria alone.

The clinical evidence of renal disease usually appeared within one year of the onset of SLE. Rarely proteinuria reached the range of 1.5 to 2.5 gm/ 24 hours.

Hill et al [51] (1978) studied 77 biopsy specimens from 59 patients with SLE and compared with the immunologic data. Excellent correlations were obtained between increasing morphologic lesions and increasing levels of DNA binding and decreasing concentrations of the third component of complement (C_3). Excellent correlation was also found with the following clinical parameters serum creatinine and urea, level of hematuria, level of proteinuria and presence of hypertension. The biopsy specimens were compared on the basis of their appearances on immunofluorescence and light microscopy, with electron microscopy as needed. They divided the biopsy specimens in to 5 principal categories, according to the location of deposits on immunofluorescence.

Class I	-	No deposits.
Class II	-	Deposits confined to the Mesangium,
Class III	-	Mesangial deposits with focal deposits along the capillary walls.
Class IV	-	Mesangial deposits with diffuse capillary deposits.
Class IV-A	-	Diffuse proliferative G.N.
Class IV B	-	Membrano proliferative G. N.
Class V	-	Diffuse sub epithelial deposits.

Appel et al [5] (1978) examined retrospectively the long term clinical course of 56 patients with SLE and these biopsies were studied by light microscopy, Electron microscopy and immunofluorescence and classified according to the system proposed by WHO which recognizes 5 classes:

I	-	Normal.
II	-	Mesangial changes.
III	-	Focal proliferative GN.
IV	-	Diffuse proliferative GN.
V	-	Membranous GN.

This WHO classification was further expanded and modified(Refer appendix). According to Sasagawa Y[85] (1993), determination of histologic class was of prognostic value regardless of the absence or severity of clinical renal disease and was the single most important prognostic indicator.

Prognosis for patients with pure mesangial lesion (II A and II B) was favorable. Patients with focal proliferative GN (III) had a clinical course similar to patients with diffuse proliferative GN (IV) frequently progressed to class IV and had an equally poor prognosis suggesting these two classes are phases of the same process. Despite the significant clinical renal disease patients with membranous lupus nephritis (Class V) had a more favorable prognosis than did patients with proliferative disease.

The relative paucity of serologic immune abnormalities in this group suggests an intrinsic difference in immune responsiveness which may be a factor determining the expression of the membranous pattern. According to them survival of patients with lupus nephritis is considerably improved compared with earlier studies and may reflect earlier diagnosis and treatment.

According to Huong DL[56] (1999), the mean age at renal disease onset was 27 years. Nephritis evolved toward end-stage renal disease despite the combined use of steroids and cyclophosphamide.

Hayslett et al [49] (1972) described the appearance of membranous lupus nephritis with epimembranous and intramembranous electron dense deposits in patients with diffuse proliferative lupus nephritis undergoing remission during treatment with azathioprine and steroids. They speculated that the change in localization of immune deposits might reflect decreased antibody production with consequent relative antigen excess and formation of small soluble circulating complexes.

Beji S et al[12] (2005) et al found that the lupus nephritis was severe in proliferative forms and age , hypertension, nephritic syndrome and initial renal failure were associated with deterioration of renal function.

MATERIAL AND METHODS

MATERIAL AND METHODS

Forty Renal biopsy specimens received in the Department of pathology, Madurai Medical College, Madurai, during the period of 2 years. The patients who presented with nephrotic syndrome and biopsied later were selected for the study.

All patients underwent routine biochemical investigations, pertaining to nephrotic syndrome, including 24 hrs urine protein, urine routine, blood sugar, blood urea, creatinine estimation.

All patients underwent ultrasound examination of the kidney, ureter and bladder prior to subjecting them for renal biopsy. All biopsy materials were collected by percutaneous renal biopsy method. This was done with patient in prone position, upon a firmly folded sheet, compressing the upper abdomen and lower ribs and fixing the kidney. Local anaesthesia was given. The lower pole is chosen for biopsy. Renal biopsy was done using Franklin's modification of Vim Silverman needle.

FIXATION AND TRANSPORT

For paraffin embedding the material was put in a container, with 10 % formalin.For light microscopic study the tissue is processed in the routine paraffin embedding method. Sections of 2 – 3 micron are taking using rotary microtome. All sections are stained with Haemotoxylin – Eosin stain.common findings and protocol are attached. (Refer appendix)

SPECIAL STAINS

For selected cases Periodic Acid Schiff stain (PAS), Masson's Trichrome stain (MTS), and Silver methanamine stains were supplemented. When a diagnosis of membranous nephropathy was made, Silver methanamine stains, MTS were used to demonstrate the basement membrane thickening. PAS was used to demonstrate increase in mesangial matrix, mesangial cellularity, basement membrane changes and to demonstate tubular casts.

IMMUNOFLUORESCENCE TECHNIQUE

Transportation

Wet gauze piece soaked in saline, in which freshly taken biopsy tissue is placed. The same kept in a plastic bag or container and sent in ice, if transportation delay is more than ½ hr.

Sample processing:

Sample is received in the department of Pathology:

A 'chuck' (tissue holder) is taken, the centre of which no '3' filter paper is placed and then the specimen is transferred from the wet saline gauze into the filter paper. The chuck with the specimen is kept inside the cold cryostat at $-30^{o}C$ for 5 minutes, for fixation.

Once the material is fixed in chuck, the tissue sections are made within cryostat at $-22^{o}C$ for 3 – 4 micron thickness (Ideal 4 micron for excellent details). The section is mounted on a slide. At least seven individual sections (only one section/ slide) are submitted for IF study.

The balance tissue put back in formalin for later use. The slides containing the sample left overnight at 22 ^{o} C, for fixation. After fixation, the studies – the site of tissue is encircled, using dark blue pencil, for easy identification of the site on which the antibody solutions have to be poured.

The Slides are marked in following order:

IgG, IgA, IgM, C3c, C1Q

(IgG – Two slides are made as artifacts uptake of IgG is high)

STEP I:

Slides are washed in PBS (Phosphate buffered saline) 3 times, rinsed and washed in 10 minutes cycle. Slides made dry.

STEP II:

Slides are kept in slide tray to which the appropriately marked, reconstituted antibodies on poured – added, in dark dust free environment, covered and kept for ½ hour contact time. The same slide, after ½ hour is re – washed.

Step I repeated – 3 washes with PBS 10 minutes x 3 cycles.

Slides are then allowed to dry.

Dried slide is mounted with glycerol in BPS (Mounting media) and the cover slip placed.

The slides are examined under – IF microscope, having mercury lamp and a blue light (NIKON – IF microscope).

IMMUNOFLUORESCENCE REPORTING:

1. GRADING :

   Grade 1 + to Grade 4 +

   LOCATION OF IF MATERIAL:-

   (Bowman's space, Basement Membrane, Mesanguim, Tubules, Interstitium & Blood vessels)

2. Granular or fine deposits.

   (Apart from the routine antisera, following may be included Fibrionogen → for transplant kidney, Albumin → for artifacts.

   Kappa and lambda chain for multiple myeloma.

OBSERVATION AND RESULTS

OBSERVATION AND RESULTS

The present study includes 40 patients of renal biopsies received in the Department of pathology, Madurai Medical College, Madurai during the period from 2003 to 2005.

Diagram (1) shows the distribution of histopathological types of nephrotic syndrome during this study period. Out of 40 patients, 2 patients were diagnosed histopathologically as Minimal change disease (MCD), 16 patients as Focal segmental glomerulosclerosis (FSGS), 7 patients as Membranous glomerulonephritis (MGN), 4 patients as IgA Nephropathy (IgAN), 3 patients as Mesangio proliferative glomerulonephritis (MSPGN), 6 patients as Systemic lupus erythematosus (LN) and 2 patients as Diffuse proliferative glomerulonephritis (DPGN).

The histological types and their incidence during the study period are given in Table (1). FSGS is the commonest type of nephrotic syndrome and the next one MGN followed closely by LN. FSGS contributes 40 % of all histological types of nephrotic syndrome whereas others are comparatively constituting 60 %. In this MGN and LN constitute 18 % and 15 % respectively.

Age distribution

Out of 40 cases 5 cases are within the age group of 12 and in the total number of five cases four are diagnosed as FSGS and one as Membranous GN. 35 cases are encountered in adults and out of which 12 cases are diagnosed as FSGS, 6 cases as MGN and 6 cases as LN. Table (2) and (3) show the Incidence of nephrotic syndrome in children and adults.

The distribution of cases according to age is shown in Table (4). In our study youngest age is eight years and oldest is seventy five years. Maximum number of cases (13 cases) were seen in fourth decade. Number of cases were drastically reduced after fifth decade. Only 2 cases are accounted from sixth to eighth decade .

Sex distribution

In our study, out of 40 cases,22 cases occurred in males (50%) and 18 cases occurred in females (40%) . Table (6) and Diagram (2).

The M: F ratio in the present study was 12: 1 in total. Male predominance was seen in MCD, MGN, and IgAN. Female predominance was seen in LN and MSPGN. The ratio was equal in DPGN and FSGS

Association with clinical presentation

When renal failure was noted at the time of biopsy in patients with nephrotic Syndrome, FSGS and LN were the common histological lesions found

When hypertension is associated with nephrotic syndrome at the time of biopsy, the commonest lesion is FSGS. Table (8) and Diagram (3) show the disease profile.

Minimal Change Disease:

In the present study 2 patients were diagnosed histopathologically as MCD. Age ranged from 20 to 35 years with a mean of 27.5 years. All patients were male. Light microscopy revealed no abnormality in glomeruli, tubules, interstitium and blood vessels {Fig (1) & Fig (2)}.

Focal segmental glomerulosclerosis:

16 patients were diagnosed histopathologically as FSGS in this study {Fig (7) & Fig (8)}. Age ranged from 8 to 45 years with a mean of 25.3 years.

M: F ratio was 1:1.

Membranous glomerulonephritis :

7 Patients were diagnosed histopathologically as membranous glomerulonephritis {Fig (3) & Fig (4)}. Age ranged from 8 – 55 years with a mean of 30.4 years and all were males.

IgA Nephropathy :

Four patients were diagnosed histopathologically as IgA Nephropathy {Fig (9) & Fig (10)}. Age ranged from 25 to 75 years. The mean age was 42.5 years. M:F ratio was 3:1. IgA Nephropathy is found in patients presenting with asymptomatic microscopic Hematuria or with recurrent episodes of gross hematuria.

Mesangio proliferative glomerulonephritis:

Three patients were diagnosed histopathologically as mesangio proliferative glomerulonephritis {Fig (14) & Fig (15)}. Age ranged from 20 to 40 years. The mean age was 25.6 years. All were females. ANA was positive in one patient.

Lupus nephritis :

6 Patients were diagnosed histopathologically as SLE . Age ranged from 18 to 45 years with a mean of 29 years. 5 patients were female. MF ratio 1:5. All the patients resented with edema feet, fever, arthralgia and decreased urine output was present in one patient. Out 6 patients, 3 were diagnosed as Class V, 2 cases as Class IV and 1 case as ClassIII LN {Fig (11), Fig (12) & Fig (13)}.

Diffuse proliferative glomerulonephritis:

2 Patients were diagnosed histopathologically as diffuse proliferative glomerulonephritis {Fig (16) & Fig (17)}. Age ranged from 20 to 40 years with a mean of 32 years. M: F ratio was 1:1 .Both patients presented with edema feet. One of the patients was hypertensive.

Immunofluorescence study:

Out of 40 cases of nephrotic syndrome, IF study was done in 10 selected cases (Table 9).2 were diagnosed as FSGS which showed diffuse glomerular mesangial deposits of IgM and C3{ Fig(18) &Fig(19} . 4 cases were diagnosed as IgA Nephropathy which revealed granular deposits of IgA within the mesangium{Fig(23) &Fig(24) }. 2 cases of SLE showed the presence of all imunoglobulins{ Fig(20)} . One case of MGN showed diffuse granular immune deposits along the GBM {Fig(21) }.One case of DPGN showed coarsely granular capillary wall staining for C3{ Fig(22) }.

DISCUSSION

DISCUSSION

Histopathologi examination of renal biopsies occupies a central position in the diagnosis and management in cases of nephrotic syndrome. Not only do they yield diagnostic information, but by proper typing, they give clues as to the disease progression and helps in planning the management of the patient.

The kidney is an efficient ultra filter and urinary protein loss is 80 – 150 mg/ day in the normal adult. 60 % of the excreted protein in filtered by glomeruli, with the remaining portion chiefly Tamm – Horsfall protein derived from tubular secretions. The nephritic syndrome comprises of heavy proteinuria, edema, hypoalbuminemia and hyperlipidemia.

Glassock defines nephrotic range proteinuria as > 3.5 gm/day although others have used 3 gm/day as the markers. Regardless of the level of protein excretion, pathological proteinuria is the defining feature and the most readily quantitated sign of nephrotic syndrome and heavy proteinuria represents profound departure from homeostasis.

It is important to recognize that the nephrotic syndrome is associated with a spectrum of primary and secondary glomerular disease. It is important to make distinction among the various causes of nephrotic syndrome, because these diverse glomerular lesions have different clinical courses, treatment and progresses. Further more distinguishing among the causes of nephritic syndrome, may aid in the development of disease specific therapies.

Clinical presentation

Glassock 44 (1995) studied more than 90 causes for the nephrotic syndrome. For the clinician, 90 percent of the cases of nephrotic syndrome in children and 70% in adults are due to three patterns of glomerular injury. Minimal change disease (MCD), focal segmental glomerular sclerosis (FSGS), and membranous glomerulonephropathy (MGN). Each of these patterns occurs in a primary or idiopathic form and in secondary forms with known causes and associations.

All the cases of nephrotic syndrome are presented with edema, hypoproteinemia and Proteinuria.

In MCD the proteinuria is highly selective- microscopic hematuria, hypertension and renal failure are uncommon as presenting signs.

But they are presented in minority or patients. In studies by Korbet et al [67] (1988), where 21% presented with hypertension and microscopic hematuria and 18% with decrease in renal function, In contrast to that, our present study shows 50% of all MCD presented with hypertension and none with microscopic hematuria or decreased renal function. Although the presentation of FSGS was similar to MCD, this is the commonest cause of steroid non responsiveness and developed a progressive course of renal insufficiency and hypertension. In our study all patients of FSGS presented with edema compatible to the studies by Beaufil et al [11] (1978).

In this 50 % of the patients presented with abdominal distension in contrast to the studies by Beaufil et al [11] (1978), where 25% of them presented with abdominal distension.

IgA Nephropathy is found in patients presenting with asymptomatic microscopic hematuria or with recurrent episodes of gross hematuria. Along with hematuria, proteinuria and renal insufficiency also occur. Gnel F et al [42] (2001) showed that the association of microscopic hematuria in IgAN was 92.8% and according to Daniel L et al [31] (2000) , hypertension with renal failure was 33.5% Similarly, in our study all with hematuria and 50% of the cases presented with hypertension and renal failure.

In case of Membranous glomerulonephritis (MGN) all patients presented with edema feet and proteinuria comparable with Noel et al [79] (1979) in which 5.8 % of patients presented with the similar features . In the previous study by Noel et al [79](1979), 25 % patients were hypertensive and 55 % showed microscopic hematuria. In contrast, in the present study, none of the patient showed microscopic hematuria and 1.4 % having hypertension.

The patients of diffuse proliferative glomeruloneprhitis presented with edema feet,proteinuria or microscopic hematuria. In the studies by Bhasin HK et al [14](1978) and Brown EA et al [16] (1979) 11% of cases presented with above features.

In our study out of three patients two were presented with hematuria and proteinuria. Unlike the present study, where only one patient was hypertensive, the above mentioned studies found a much higher prevalence of hypertension associated with this condition. In the present study. ASO titres were elevated only in 50 % of the patients, in contrast to the previous study by Herdson et al[50] (1966), who found elevated ASO titres in all patients.

In cases of lupus nephritis (LN) Proteinuria was detected in all patients of our study. ANA was positive in all patients, similar to the findings of Baldwin [7] (1977). Two patients (33 %) in the present study were hypertensive which is in accordence with the findings of Beji S et al [12] (2005) of whom found 32.3% of the patients to be hypertensive.

Age Incidence:

In cases of minimal change disease of our study, out of 40 cases 2 cases were diagnosed histopathologically as Minimal Change Disease. The age incidence in the present study was 20 – 35 years, in contrast to the studies done by Korbet et al [67] (1988) which showed most of the patients to be in the in the age of 40. In present study MCD is not encountered because it is more common in children below 8 years .The youngest age group in our study is 8 years and hence the true statistical incidence in children could not be commented, as the number of pediatric renal biopsies were low.

In case of FSGS age incidence is ranging from 8 to 45 years with a mean of 25.3. In contrast, in the study by Beaufils et al [11] (1978) mean age was 30 years.

In cases of IgA Nephropathy age incidence is ranging from 25 to 75 years with a mean age was 42.5 years, in contrast to the study by Tang Z et al [90] (2002) which showed the mean age of 28.5 years.

In cases of MGN age incidence ranges from 20 to 55 years with a mean of 30.4 years in the present study. The study by Azad NS et al [5] (2004) showed mean age of 29 years.

The age incidence in the cases of DPGN in the present study was 24 to 40 in contrast to the study by Herdson et al [50] (1966) which showed the age range of 12 to 60 years.

In cases of MSPGN in our study age ranging from 20 – 40 years, with a mean age of 25.6 in contrast to the study by Brown EA et al [16] (1979) wherein age range was 2 to 65 years.

Age incidence in LN ranging from 18-46 years which is comparable to the studies by Baldwin [7] (1977).

Sex Incidence:

MCD showed a male predominance in the present study because of limited sampling in children which is in contrast to the studies by Korbet et al [67] (1988) which showed female predominance. The M: F ratio in cases of FSGS of our study was 1:1 in contrast to the study by Beaufils et al [11] 1978 showed male predominance.

In cases of MGN, our study showed male predominance in contrast to the study of Azad NS et al [5] (2004) which had ratio of 2.2:1. In cases of IgA N, the male to female ratio was 3:1 similar to the study of Daniel L et al [31](2000.) In cases of MSPGN M: F ratio was 0:3 contrast to the male predominance in study by Brown et al [16] (1979). All the cases of LN showing female predominance which is comparable to the studies by Baldwin [7] (1977).

Statistical evaluation of different types of glomerulo nephropathies:

The present study included 40 cases of renal biopsies in cases of Nephrotic syndrome received in the Department of pathology, Madurai Medical College, Madurai in 2 years. The most common histological entities diagnosed in present study including children and adults were focal segmental glomerulosclerosis (40 %) and the next two are membranous glomerulo nephritis (18 %) and systemic lupus erythematosus 15 %.

In the study of Glassock [44] (1995) 90 % cases of Nephrotic Syndrome in children and 70 % cases in adult are due to the following three patterns MCD, FSGS and IGA. Similar to that out study shows 100 %. Cases of Children and 63 % adults are due to these lesions.

According to study Kumar J et al [70] (2003), nephrotic syndrome in cases of children the mean age was 7.9. The commonest histopathologically subtype was FSGS (38%). In children under the age of 8, MCD was the most common entity, whereas FSGS predominated in children with age at onset greater than 8 years. Similar to that, our study encountered cases with lowest age of 8 years in children and the commonest subtype as FSGS (80%).

According to the studies by Cruz HM et al [30] (1989), nephrotic syndrome in adults, the commonest type was FSGS followed by MGN. Similar to that, FSGS (34%) followed by MGN (17%) are the most frequently occurring types in our study.

Clinico pathological correlation:

Idiopathic nephrotic syndrome is a common problem seen in children and adults. Apart from clinical and biochemical investigations, histopathological diagnosis is very useful marker in predicting the response to therapy and long term renal outcome. Histopathologic study of primary glomerulopathies in adults by Mazzarolo Cruz et al [30] in 1996 found that FSGS followed by MGN were the commonly encountered lesions and prevalences were similar in both sexes with mean age of 35 years.

In recent study from CMC, Vellore by Balakrishnan et al [6] in 2002 MGN and FSGS are commonly associated with adult nephrotic syndrome.

A recent study was done in pediatric age group by Kumar J et al [70] in 2003 showed that in children under 8 years of age, MCD was the most common entity whereas FSGS predominated in children at onset greater than 8 years. In comparison, our study shows FSGS to be commonly associated with nephrotic syndrome both in adult and children.

With regards to sex distributions, FSGS and DPGN are found equally in both sexes MCD and MGN are seen only in males and MPGN found only in females in our study. LN is more common in females and IgAN is common in males.

According to age distribution, FSGS is the commonest cause of nephrotic syndrome under 40 years and MGN in the commonest cause of nephrotic syndrome beyond 40 years.

In our study, when renal failure is present at biopsy, FSGS followed by LN and IgAN are the common diagnosis arrived. When there is no renal failure at biopsy FSGS followed by MCN are found. FSGS and LN are commonly associated with hypertension.

Light microscopy and immunofluorescence correlation:

Many believe that the interpretation of renal biopsy is extremely difficult and it has become more complex over the years but now the diagnosis of renal biopsy specimen is based on the same foundation of careful observation, clinicopathological correlation and Immunofluorescence study.

This is largely due to the fact that different patterns of reactivity in glomerular disease are easier to recognize by IF microscopy.

Out of 40 cases 10 cases were subjected to IF study. In present study out of 40 cases, 2 cases were diagnosed as Minimal change disease. In case of Minimal change disease, Light microscopy revealed no abnormality of glomerular, tubules, interstitial and blood vessels [Fig (1) &Fig (2)]. IF studies are almost invariably negative for immunoglobulin and complement.

In present study, out of 40 cases, 16 cases were diagnosed histopathologically as FSGS. In cases of FSGS light microscopy revealed focal and segmental sclerosis of glomeruli in all cases with slight mesangial cell hypercellularity. Capillary lumina were patent [Fig (7) & Fig (8)]. All the features are in concordance with previous study conducted by Beaufils et al [11] (1978). Out of 16 cases 2 cases were subjected to IF study. In IF study diffuse glomerular mesangial deposits of IgM andC3 (Fig 18) & Fig(19)} are seen in low intensity similar to the study of Agarwal SK [2] (1993).

7 cases were diagnosed histopathologicalIy as Membranous nephropathy. Light microscopy showed moderate thickening of basement membrane [Fig (3) &Fig (4)] in three cases (43 %) & mild thickening in four cases (57 %).

In contrast to the study by Tooth et al [91] (1992) showed 52% of cases with moderate thickening. In present study there was no increase in mesangial cellularity in any of the cases in contrast to study by Danilewiez & Wgrawska [32] (1995) in which glomerular cells were significantly increased. In IF study diffuse granular pattern of immune reactants that follows the GBM and spares the mesangium (Fig 21). IgG is the predominant Ig & C_3 which may present in 5 to 50 % of cases. This is similar to the study of Berger et al [13] (1969) who found that these sub-epithelial deposits always fix anti IgG serum

Four cases were diagnosed as IgA nephropathy in which mesangial hyper cellular is the most frequent light microscopic pattern [Fig (9)]. Extra capillary proliferation in some cases going for crescent formation either or cellular or fibrous [Fig (10)].IF study was done in all the 4 cases. In IF study, granular deposits of IgA & C_3 within the mesangium {(Fig 23) & Fig (24)} is the regular and consistent feature of IgA N. This is similar to the study of Berger & Hinglais et al [13] (1968).

Six cases were diagnosed as Lupus nephritis. In present study light microscopy showed enlarged and hypercellular glomeruli in all patients and increased endothelial and mesangial cell proliferation in 2 cases [Fig (11)]. Basement membrane was thickened [Fig (12) &Fig (13)] in all patients and 1 patient showed fibro epithelial cresents in occasional glomeruli. Out of 6 cases, 3 cases were diagnosed as class V (Diffuse membranous GN), 2 cases as class IV (Diffuse GN) and 1 case as class III (Focal segmental GN).The commonest histological type of LN in our study is class V. Out of 6 cases 2 cases were subjected to IF study and confirmed with the presence all immunoglobulins .All the findings are similar to the studies by Baldwin [7] (1977). Classically Lupus nephritis is characterized by a full house sign of immune stain with IgG, IgM, IgA, and IgE with C1q, C_3 and $C_{4\ Fig}$ (20). In Practice IgG is the most common Ig detected according to the study of Bannister. K.M. [9] (1983).Tubular basement membrane also showed staining for IgG as per the study of Jennete60 (1983).

3 cases were diagnosed histopathologically as MSPGN in the present study. Light microscopy showed hypercellular glomeruli with increased mesangial cell proliferation Fig [(14)&Fig(15)] in all patients. Capillary Lumina were patent in all patients. These findings were comparable to the study by Brown et al [16] (1979).

In present study out of 40 patients. 2 patients were diagnosed as DPGN Light microscopy showed hypercellular glomeruli with increased mesangial and endothelial cell proliferation [Fig (16)] in all patients. Lobularity of glomeruli was exaggerated and capillary lumina were narrowed in all patients. These findings are similar to the study Herdson et al [60] (1966). Neutrophils were found in the glomeruli [Fig (17)] in most of the patients in the present study, similar to the study by Herdson et al [50] (1966) an IF study showed global coarsely granular capillary wall staining for C3.(Fig22)

COMPARATIVE EVALUATION WITH OTHER CENTRES:

The causes of Idiopathic nephrotic syndrome vary among different countries.

In a recent study from CMC, Vellore by Balakrishnan et al [6] in 2002. Membranous glomerulonephritis and focal segmental glomerulosclerosis are commonly associated with adult idiopathic nephrotic syndrome. In comparison, our study also shows FSGS & MGN to be commonly associated with nephrotic syndrome.

In children the causes of Idiopathic nephrotic syndrome due to three patterns of glomerular injury: MCD, FSGS and MGN. A study from CMC Vellore in 2002 showed MCD is the commonest cause. In comparison our study shows FSGS is the commonest cause associated with nephrotic syndrome in children.

In adults the nature of renal lesions in idiopathic nephrotic syndrome varies among different countries. In an Indian study, by Hague et al [47] in 2001, MPGN was 42 %; MSPGN was 18.4 % among all idiopathic nephrotic syndromes in adults. In a recent study from CMC Vellore 2002, Focal segmental glomerulo sclerosis was commonly associated with adult idiopathic nephrotic syndrome. Cruz et al [3o] and Mazzarollo et al [78] reported on the patterns of glomerulonephritis in western countries in 1989 and 1996 respectively.

They found that FSGS was commonly associated with adult nephrotic syndrome. In comparison, our study shows FSGS and Membranous glomerulo nephritis to be commonly associated with nephrotic syndrome.

Summary and conclusion

CONCLUSION OF THE STUDY:

1. FSGS & membranous glomerular nephritis are the common causes of Nephrotic Syndrome

2. FSGS and Diffuse proliferative glomerulonephritis are common in both the sex.

3. Mesangioproliferative glomerulonephritis is found predominantly in females.

4. In patients with SLE which is commoner in females, Class IV and Class V lesions of lupus nephritis were predominantly found.

5. If hypertension and renal failure are present at the time of renal biopsy, Ig A nephropathy and lupus nephritis are the common histopathological lesions noted.

6. Immunofluorescence examination is very valuable in evaluation of renal biopsies especially in the conclusive diagnosis of IgA Nephropathy.

TABLE 1 :INCIDENCE OF RENAL LESIONS ENCOUNTERED

Types	No. of Patients	Percentage
Minimal Change Disease (MCD)	2	5 %
Focal Segmental Glomerulo Sclerosis (FSGS)	16	40 %
Membranous Glomerulo Nephritis(MGN)	7	18 %
IgA Nephropathy (Ig AN)	4	10 %
Mesangio Proliferative Glomerulo Nephritis (MSPGN)	3	7 %
Systemic Lupus Erythematosis (LN)	6	15 %
Diffuse Proliferative Glomerulo Nephritis (DPGN)	2	5 %
TOTAL	40	100%

Diagram 1: Distribution of Histopathological types - Nephrotic Syndrome

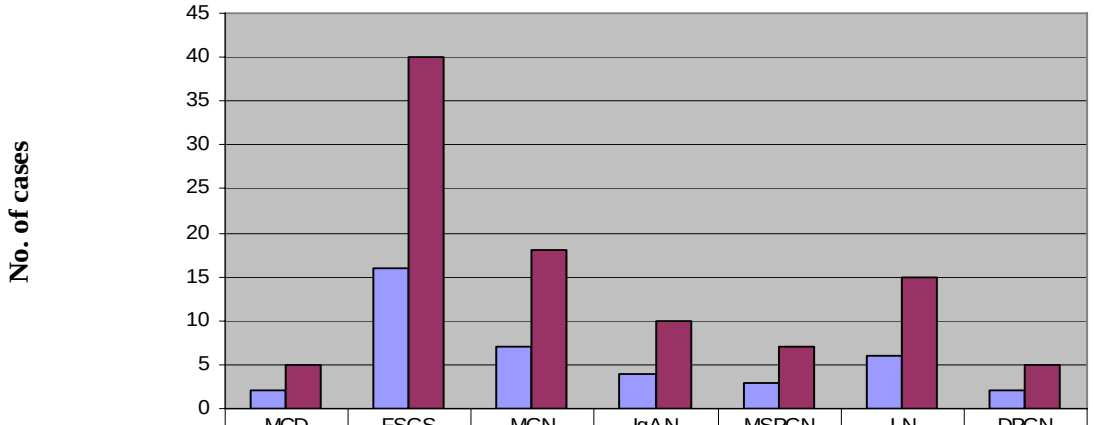


TABLE 2:AGE INCIDENCE OF NEPHROTIC SYNDROME

Age in years	No. of cases	Male	Female
< 12 years	5	3	2
12- and above	35	19	16

Diagram 2: AGE INCIDENCE OF NEPHROTIC SYNDROME

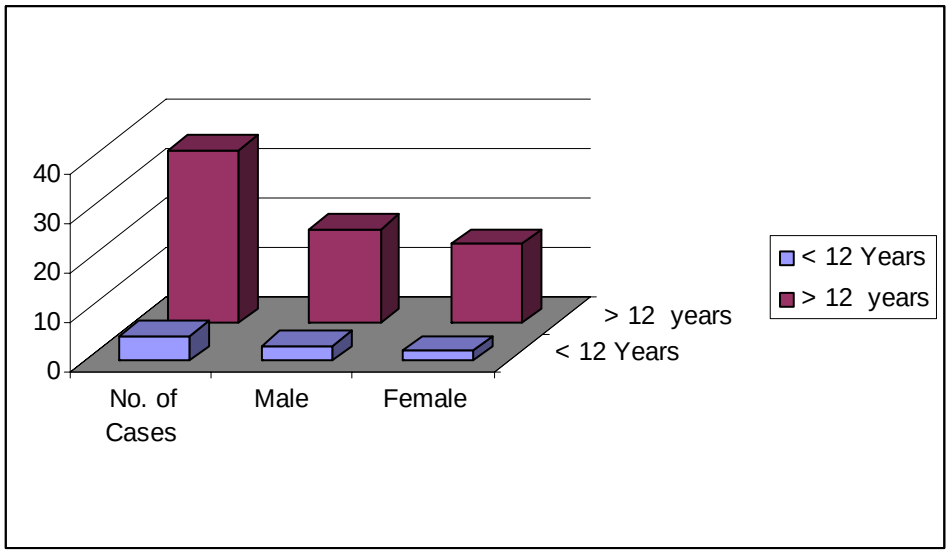


TABLE 3: RENAL HISTOLOGICAL TYPES & INCIDENCE IN CHILDREN & ADULT

Types of GN	CHILDREN		ADULTS	
	No. of Cases	**Percentage**	**No. of Cases**	**Percentage**
MCD	-	-	2	7%
FSGS	4	80%	12	34%
MGN	1	20%	6	17%
IgAN	-	-	4	11%
MSPGN	-	-	3	8%
SLE	-	-	6	17%
DPGN	-	-	2	7%

TABLE 4: DECENNIAL AGE DISTRIBUTION

No.	Age Group in Years	No. of Cases	Percentage
1	Upto10 years	4	10 %
2	11 – 20 years	8	20 %
3	21- 30 Years	10	25 %
4	31 – 40 Years	13	32.5 %
5	41 – 50 years	3	7.5 %
6	51 – 60 years	1	2.5 %
7	61 – 70 years	-	-
8	71 – 80 years	1	2.5 %
	Total	40	

youngest age - 8 years.
Oldest age - 75 years.

TABLE 5: DECENNIAL AGE DISTRIBUTION

IN DIFFERENT TYPES OF GLOMERULOPATHIES STUDIED

Type	**Age in years**								Grand Total
	0 to 10	11-20	21-30	31-40	41-50	51-60	61-70	71- 80	
MCD		1		1					2
FSGS	3	3	3	6	1				16
MGN	1	2	1	1	1	1			7
IgA			1	2				1	4
SLE		1	3	1	1				6
MSPGN		1	1	1					3
DPGN			1	1					2
%	10	20	25	32.5	7.5	2.5	--	2.5	100%

TABLE 6: SEX DISTRIBUTION IN PRESENT STUDY

Type	Male	Female	M:F Ratio
MCD	2	0	2:0
FSGS	8	8	1:1
MGN	7	0	7:0
IgAN	3	1	3:1
LN	1	5	1:5
MSPGN	0	3	0:3
DPGN	1	1	1:1

TABLE 7: NUMBER OF PATIENTS WITH OR WITHOUT RENAL FAILURE AT THE TIME OF BIOPSY

S.No.	AT BIOPSY		
	DISEASE PROFILE	RENAL FAILURE	NO RENAL FAILURE
1	MCD	0	2
2	FSGS	5	11
3	IgAN	2	2
4	MGN	1	6
5	DPGN	1	1
6	MSPGN	1	2
7	LN	3	3
	Total	13	27

TABLE 8: CASES CLINICALLY PRESENTED WITH HYPERTENSION WHEN BIOPSIED

S.No.	TYPES OF GN	HYPER TENSION	
		HYPER TENSION (+)	HYPER TENSION (-)
1	MCD	1	1
2	FSGS	4	12
3	IgAN	2	2
4	MGN	1	6
5	DPGN	1	1
6	MSPGN	2	1
7	LN	2	4
	TOTAL	13	27

DIAGRAM 3 DISEASE PROFILE IN RELATION TO HYPERTENSION WHEN BIOPSIED.

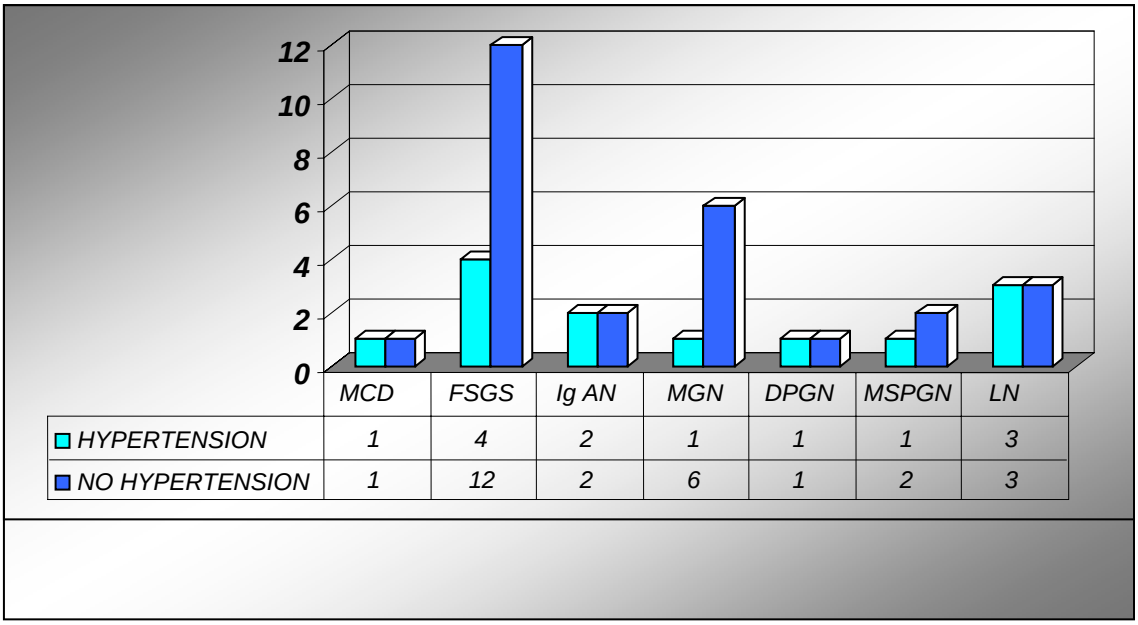


TABLE 9： **IMMUNOFLUORESCENCE STUDY**

No of cases	Path No	Types of GN	Immunofluorescence study findings
	1994/03	FSGS	Granular deposits of C3c in focal segmental distribution & mesangial granular deposits of IgM.
2	3558/03	IGAN	Mesangial granular deposits of IgA, C3c & IgM.
3	8996/03	DPGN	Abundant peripheral and mesangial granular deposits of C3c and moderate amounts of IgG and IgM in the glomeruli.
4	630/05	FSGS	Mesangial granular deposits of IgM.
5	610/05	IGAN	Mesangial granular deposits IgA, C3c and IgM.
6	3924/05	MGN	Peripheral granular deposits of IgG,C3c and C1q.
7	6225/05	SLE(LN)	Peripheral and mesangial granular deposits of all classes of Ig & complements.
8	16057/05	IGAN	Mesangial granular deposits of IgA, C3cand IgM.
9	1267/05	IGAN	Mesangial granular deposits of IgA, C3c and IgM.
10	724/05	SLE(LN)	Peripheral and mesangial granular deposits of all classes of Ig and complements.

TABLE 10 -: CAUSES OF CHILDHOOD NEPHROTIC SYNDROME – COMPARISON WITH OTHER CENTRES

S.No.	Diagnosis	CMC Vellore (2002)	KUMAR et al (2003)	Present Study
1	MCD	47.2 %	32%	-
2	MGN	-	2%	20%
3	FSGS	12.5 %	38%	80 %
4	PGN	8.8 %	15%	-
5	SLE	3.8 %	-	-
6	MPGN	11.3 %	11%	-
7	OTHERS (Adults)	16.4%	2%	-

TABLE 11: Types of Renal lesions found in adult nephrotic syndrome (Comparison with other centers)

S.No.	Diagnosis	Cruz et al (1989)	Mazzarallo et al(1996)	Haque et al(2002)	CMC Vellore (2002)	Present Study
1	MCD	5.1 %	5.3%	-	8.7 %	5.7 %
2	FSGS	37.1 %	43.2	2.6 %	22 %	17 .1%
3	MGN	16.2 %	20.4	15.8 %	11 %	34.2 %
4	IGA N	8.6 %	10.2 %	-	-	11.4 %
5	SLE	-	-	-	-	17.1 %
6	MSPGN	-	2.9%	18.4 %	-	8.5 % 5.7 %
7	DPGN	4.6 %	1.9%	5.2 %	40.7 %	-
8	MPGN	6.1 %	14.1 %	42 %	-	
9	Others (Children)	22.3%	2.0%	16%	17.6%	0.3%

ANNEXURE – I (PROFORMA)

ANNEXURE-1

PROFORMA

DEPARTMENT OF PATHOLOGY,
MADURAI MEDIAL COLLEGE, MADURAI.

Name Age : Sex: I.P. NO.
Biopsy No.

Presenting C/o: Duration:
Drug Intake:

Past History: Hypertension
Diabetes Mellitus
Skin lesion.
Urinary Tract Infection
Joint pain

Family History
Diabetes mellitus
Hypertension
Other Renal Disease.

Personal History: Smoker
Alcoholic

Attained Menarche
Attained Menopause
No of child birth
History of abortions
LCB

O/E: BP - Pulse
Pedal edema
Facial puffiness
Skin lesions

CVS
RS
Abd
CNS
PR
PV

Laboratory Investigations:

Urine : Albumin: Sugar: Deposits:

24 hrs. Urinary Protein:

Blood : Urea : Sr.Creatinine : Sugar:

Sr. Total Protein:

C3, ANA

USG ABDOMEN

KIDNEY BIOPSY: Light Microscopy

Glomeruli

Tubules

Interstitium

Blood Vessels

Special stains.

Immunofluorescence

Final Diagnosis.

COMMON GLOMERULAR LESIONS ASSOCIATED WITH NEPHROTIC SYNDROME

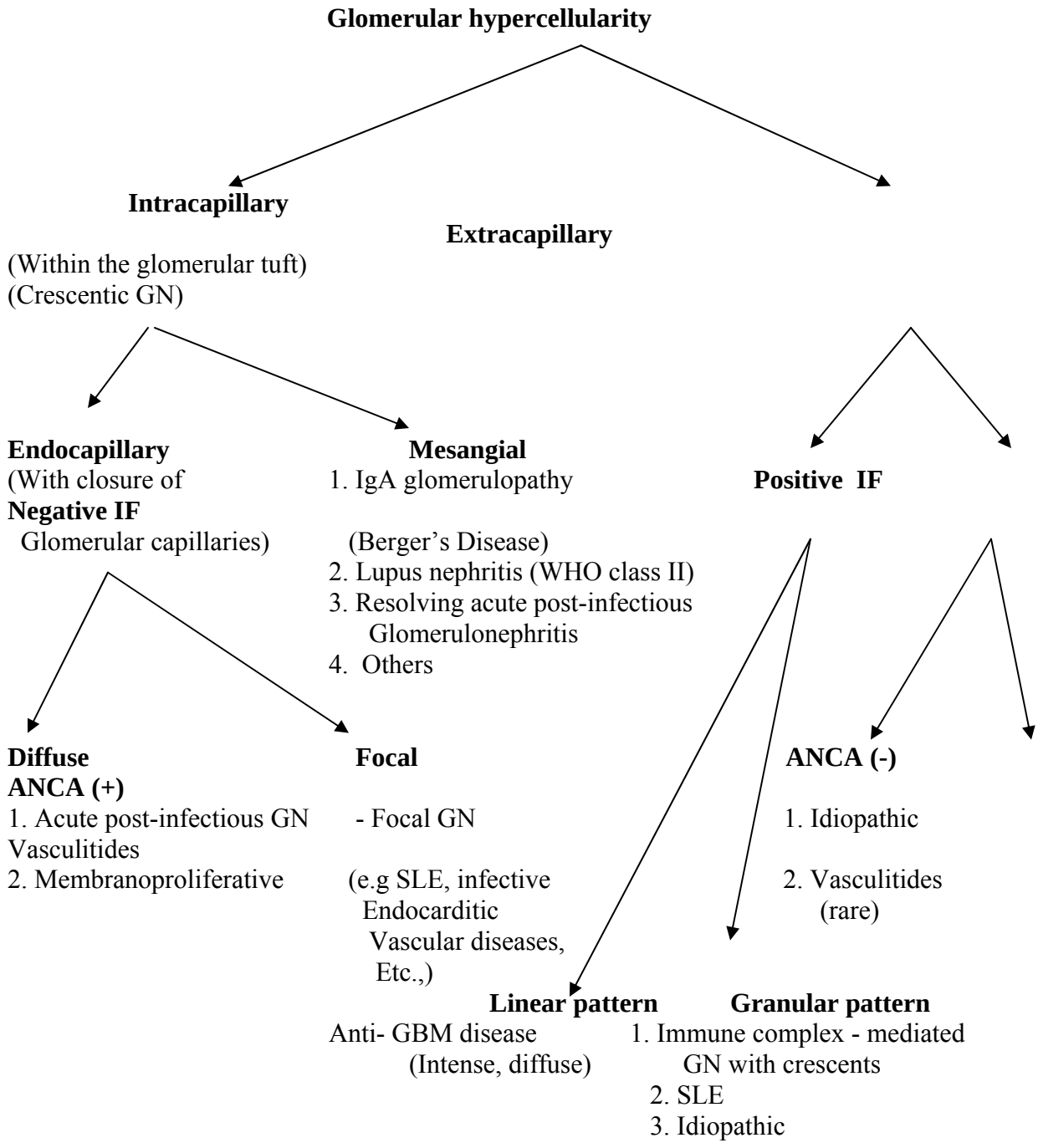
DISEASE	LIGHT MICROSCOPY	IMMUNOFLUORESCENCE
1. Minimal change disease	Minimal or no mesangial Prominence; Glomerular Visceral epithelium may be prominent with tadpole shaped Nuclei.	Usually negative for Immunoglobulins and C_3
2. Diffuse Proliferative Glomerulonephritis	Mesangial hypercellularity.	Mesangial deposits of IgM with or without C_3.
3. Membranous Glomerulonephritis	Uniform capillary wall thickening **(PAS – Positive) v**acuolated appearance of tangentially cut portions of TBM, Spike porojections of BM.	Granular deposits along capillary wall of IgM and C_3 sometimes IgM and IgA
4. Membrano Proliferative Glomerulonephritis	Type I : Endo capillary cellular increase, principally of mesangial Cells, mesangial expansion, increased matrix, characteristic splitting or reduplication of basement membrane (double contoured basement membranes) Type II: Dense intra membrane changes/ deposits, deposits of basement membrane, thick, bright acidophilic, refractile basement membrane (PAS – Positive). Segmental thickening of capillaries ("string of sausages)	Diffuse, coarse or fine, granular staining of the peripheral capillary wall and mesangium for both immunoglobulins and C_3 C3 demonstration along the capillary wall. Pattern of staining – linear, pseudo linear, smooth granular and nodular.
5. IgA Nephropathy	Widening of mesangium by increased matrix and hyper cellularity. Necrotizing or sclerosing glomerular changes.	Diffuse mesangial deposits of IgA (often associated with IgG and C 3)
6. Focal Segmental Glomerulosclerosis	Focal and segmental mesangial sclerosis.	Non specific trapping of IgM and C3

7. Amyloidosis	Mesangial and vascular deposits of congo red – positive material with green birefringence.	Amyloid AA and light chains.
8. Light chain Nephropathy	Mesangial widening and deposition of PAS – positive material.	Peripheral and mesangial kappa and Lamda light chains.
9. SLE	Wire loop lesion, tram track appearance. The major histologic abnormalities of the glomerulus include immune deposits, glomerular proliferation influx of leukocytes glomerular necrosis and scarring. Glomerular proliferation may be mesangial, endocapillary and extracapillary. deposits seen with special stains – appear red with Trichrome stains, against the blue glomerular matrix, appear pink red with trichrome stains, against the blue glomerular matrix, appear pink red with Jones – methanamine siliver, subendothelial deposits are regularly seen in Class III and Class IV.In class IV SLE, they are large enough to completely involve the peripheral circumference of glomeruli referred as "wire – loops refractile thickening of glomerular capillary wall other findings include cellular crescents, vasculitis and tubulo interstitial changes like inflammation and edema.	Immune deposits of IgG, IgM, IgA, C3, C1q is found in mesangium sub epithelium and sub endothelium areas called "FULLHOUSE' Immunofluroscence.

ALGORITHMS FOR MORPHOLOGIC INTERPRETATION OF GLOMERULAR

PATTERNS:

A. Is there glomerular hypercellularity?
B. Are there glomerular capillary light microscopic changes?



ALGORITHMS FOR INTERPRETATION OF IMMUNOFLUORESCENCE MICROSCOPY

Negative Immunofluorescence
Positive Immunofluorescence

Positive Immunofluorescence

Non-specific deposition/ absorption Of Immunoreactants

Specific pattern

A.Glomerular
-Sclerosis (Global or segmental) (Usually IgM/ C3)
-Linear – GBM (diabetic nephropathy)
-Glomerular visceral epithelial cells

B.Tubulo – interstitial
-Linear – TBM (diabetic nephropathy, Tubular atrophy)
-tubular epithelial cells (Proteinuric States)
-tubular casts (usually IgA, IgM, kappa And lambda)

C.Vascular
- Arteries / arterioles (usually IgM And C3)

A. Glomerular
1. Linear (GBM)
- Anti – GBM disease
- DDD
- Light/ heavy chain Deposition disease

2. Granular
- Mesangial
- IgA GP
- Lupus nephritis
- Capillary wall
- Membranous GP
- MPGN
- Acute post – infectious GN
- Combined
- Lupus nephritis
- MPGN
- Acute post-infectious GN

3. Nodular
- Mesangial
- DDD
- Amyloid

B. **Tubulo-interstitia**l
1. Linear (TBM)
- Anti-GBM disease
- DDD
- Light/ heavy chain deposition disease.

2. Granular (TBM)
- Lupus nephritis
– Acute post-Infectious
- Tubulo-interstitial nephritis
- Others (rare)

3. Tubular casts
Myeloma cast Nephropathy

4. Interstitium
Lupus nephritis.

C. **Vascular**
1. Granular lupus nephritis

Evaluation of Kidney Biopsy – Recommended protocol

LIGHT MICROSCOPY:

Because of its small size, the kidney biopsy specimen requires a systematic, detailed and analytic approach for accurate evaluation. All stained sections on each slide are surveyed to deter in the adequacy of the specimen, to obtain an idea of distribution of the lesion and their characteristics in various renal structures at the different levels of the block and to select for each stain, the most representative sections for detailed study.

ADEQUACY OF THE SPECIMEN:

Minimum 5 – 10 glomeruli essential (In disorders of irregular or crescentic proliferation minimum 10 glomeruli needed).

Following Renal Compartments are systematically analysed:

G L O M E R U L I .

1. Glomeruli – Distribution of lesions are categorized as Segmental Diffuse, Focal and generalised.

Individual Glomeruli

i) Segmental – Lesion affects only part of tuft (Less than 50 %)

ii) Global – Lesion affects the entire tuft.

All Glomeruli

i) Focal – lesion affects less than 50 % of the Glomeruli seen in section.

ii) Diffuse – When the lesion affects all the Glomeruli.

Following changes noted in Glomerular morphology:

1. Size.
2. Lobulation
3. Necrosis.
4. Sclerosis.
5. Hyalinization.
6. Cellularity.
7. Changes in Glomerular cells – swelling, hypertrophy, multinucleation.
8. Basement membrane (Ideal silver stains) – examined for increased or decreased thickness, irregular contours, wrinking, splitting, reduplication, rarefaction, presence of deposits in the lamina densa.
9. Protein deposits (mesangial, subendothelial, subepithelial and intramembranous)
10. Capillary lumen – narrowed or dilated partialy or completely obstructed by thrombi.
11. Bowman's space and capsule – crescents – fibroepithelial, fibrous crescents, Any dilatation or collapse.

* T U B U L E S *

TUBULES ARE EXAMINED FOR:

1. Atrophy – Focal or diffuse. If associated with interstitial edema, fibrosis and inflammation.
2. Hypertrophy – compensatory or diffuse.
3. Necrosis.
4. Degenerative changes (pigmentary, inclusions, calcific, crystals, glycogenosis)
5. Tubular basement membrane changes – thickening, deposits.
6. Tubular contents – hyaline, hyaline – granular, waxy and broad tubular casts)

INTERSTITIUM

1. Fibrosis, edema and hemorrhage.
2. Inflammation.

* A R T E R I E S *

(Preglomerular, postglomerular arterioles, Interlobular arteries)

Wall thickness to lumen size assessed.

0 Normal wall thickness that is less than the diameter of the lumen.

1 + Increased wall thickness but to a degree that is less than the diameter of the lumen.

2 + Wall thickness that is equal to the diameter of the lumen.

3 + Wall thickness that is greater than the diameter of the lumen.

4 + Wall thickness that far exceeds the diameter of the lumen with extreme luminal narrowing or occlusion.

SPECIAL STAINS USED ARE:

A. PERIODIC ACID SCHIFF STAIN:

Principle:

Periodic acid oxidizes compounds having free hydroxyl a group resulting in a dialdehyde which is demonstrated with is demonstrated with a Schiff reagent. Periodic acid will cleave the carbon – carbon bonds where these carbon atoms have adjacent hydroxyl groups (1:2 glycol groups) or adjacent hydroxyl and amino groups (1:2 amino hydroxyl groups) and a dialdehyde structure is produced.

Solutions required:

1. Periodic acid:
   Periodic acid – 1 g
   Distilled water - 100 ml.

2. Schiff reagent:
   Basic fuchsin – 1 g.
   Distilled water – 100 ml.
   Sodium Metabisulphite – 2 g.
   Concentrated hydrochloric acid – 2 ml.
   Activated charcoal - 2 g

3. Mayer's haematoxylin:
   Haematoxylin powder – 1 gm
   Aluminium sulphate - 50 Gms.

Schiff Reagent:

Boil 200 ml distilled water in a conical flask and remove from heat add 1 gram of basic fuchsine, mix well. Cool it to 50 degree centigrade (check with thermometer) adds sodium Metabisulphite. Let it cool to room temperature, and then add 20 ml 1 % hydrochloric acid. Mix it and then add 2 g charcoal. Pour this in carbon wrapped bottle and refrigerate for 24 hours. Then take it and filter. Store filtrate in carbon wrapped bottle in fridge.

METHOD:

- Bring sections to water.
- Oxidize in 1 % periodic acid for 5 minutes.
- Wash in water.
- Treat with Schiff reagent for 10 – 15 minutes.
- Wash in water for 10 minutes.
- Counterstain with Mayer's haematoxylin for 5 minutes.
- Wash in water.
- Dehydrate, clear and mount.

RESULTS:

PSA Positive substances – Magenta (Purplish red)
Nuclei – Blue.

B. RETICULIN STAIN (GOMORI's RETICULIN METHOD)

Silver solution:

To 4 parts of 10 % aqueous silver nitrate add 1 part of 10 % potassium hydroxide. Allow the deposit to settle, remove the supernatant and wash the deposit twice with distilled water. Make upto original volume with distilled water. This step helps to give a cleaner background. Add fresh strong ammonia (sp. Gravity 0.88) drop by drop until the deposit is just dissolved. Carefully add 10 % silver nitrate drop by drop until the solution takes on a faint sheen. Make solution upto twice its original volume.

METHOD:

- Bring sections to water.
- Oxidize in 1 % potassium permanganate for 1 minute.
- Rinse in tap water.
- Bleach in 5 % oxalic acid for 1 minute.
- Rinse in tap water.
- Sensitize in 2.5 % Iron alum for 1 minute.
- Wash well in tap water, rinse in distilled water.
- Impregnate in silver solution for 4 minutes.
- Rinse rapidly in distilled water.
- Reduce in 10 % aqueous formalin for 1 minute.
- Wash well in tap water, rinse in distilled water.
- Fix in 5 % thiosulphate (hypo) for 1 minute.
- Wash in water.
- Dehydrate, clear and mount.

RESULTS:

Reticulin fibres – black.

Collagen, cells including nuclei – purple grey if toned.

WHO MORPHOLOLGICAL CLASSIFICATION OF LUPUS NEPHRITIS

I. Normal Glomeruli
   A. Nil (by all techniques)
   B. Normal by light microscopy but deposits by electron and Immunofluorescence Microscopy.

II. Pure mesangial alterations (Mesangiopathy)
   A .Mesangial widening and / or mild hypercellularity (+)
   B. Moderate hypercellularity (+ +)

III. Focal segmental glomerulonephritis (associated with mild or moderate Mesangial alterations)
   An .Active necrotizing lesions.
   B. Active and sclerosing lesions
   C. Sclerosing lesions.

IV. Diffuse glomerulonephritis (severe, mesangial, endocapillary or Mesangiocapillary proliferation and/ or extensive subendothelial deposits) Mesangial deposits are present invariably and subepitjelial deposits often and may Be numerous.
   A.Without segmental lesions.
   B. With active necrotizing lesions.
   C. With active and sclerosing lesions.
   D. With sclerosing lesions.

V. Diffuse membranous glomerulonephritis.
   A. Pure membranous glomerulonephritis.
   B. Associated with lesions of Category II (a or b)

VI. Advanced sclerosing glomerulonephritis.

ANNEXURE – II BIBLIOGRAPHY

ANNEXURE – II

BIBLIOGRAPHY

1 Addis T. Oliver J: The Renal lesions in Bright 's disease. P. Hoeber, New York 1931. Renal Biopsy interpretation 1996. P No. 2-18.

2.Agarwal SK, Dash SC, Tiwari SC, Bhuyan UN. Idiopathic adult focal segmental glomerulosclerosis: a clinicopathological study and response to steroid: Nephron. 1993;63(2):168-71.

3. Alwall N: Aspiration biopsy of the kidney including a report of a case of amyloidosis diagnosed through aspiration biopsy of the kidney in 1944 and investigated at an autopsy in 1950. Acta Med Sc and 43:430, 1952.

4. Appel GB, Silva FG. Pirani (T. Melizer H. Estes D: Renal involvement in systemic lupus erythematosus (SLE :) A study of 56 patients emphasizing histologic classification. Medicine (Baltimore) 57:371-410.1978.

5.Azad NS, Muzaffar S, Pervez S, Ahmed A, Rabbani A, Ali A: Demographic and Clinicopalthologic features of membranous glomerulonephritis: J Coll Physicians Surg Pak. 2004 Jan;14(1):35-8.

6. Balakrishnan N, John GT, Vishalakshi J, Talaulikar GS, Thomas PP, Jacob CK.Spectrum of biopsy proven renal disease (BPRD) and changing trends: 33rd Annual Conference of Indian Society of Nephrology CN-0-17-1, 2002.

7. Baldwin DS, Luck MC, Lowenstein J, and Gallo GR: Lupus nephritis. Clinical course as related to morphologic forms and their transitions: Am J Med.1977 Jan; 62(1):12-30.

8. Ball RP: Needle aspiration biopsy. J Ten Med Assoc 27: 203, 1934.

9.Bannister km, Howarth GS, Clarkson AR, Woddrotte AJ: Glomerular IgG subclass distribution in human glomerulo nephritis. Clin Nephrol 19:161, 1983.

10.Bazzi C, Retrni C, Rizza V, Arrigo G, D' Amico G. A modern approach to selectivity of proteinuria and tubulointerstitial damage in nephritic syndrome: Kidney Int . 2000 Oct : 58(4): 1732 -41.

11.Beaufils H, Alphonse JC, Guedon J, Legrain M. Focal glomerulosclerosis: natural history and treatment. A report of 70 cases: Nephron. 1978;21(2):75-85.

12.Beji S, Karroud H, Ben Moussas F, Abderrahim E, Goucha R, Ben Hamida F, Hedri H, EI Younsi F, Ben Abdallah T, Kheder A, Ben Maiz H. (Lupus nephritis: 211 cases): Rev Med Interne.2005 Jan;26 (1):8-12.

13.Berger J. Hinglais N: Les depots intercapillaries d' IgA-IgG. J Urol Nephrol (Paris) 74:694-695.1968.

14.Bhasin HK, Abuelo JG, Nayak R, Esparza AR. Mesangial proliferative glomerulonephritis: Lab Invest. 1978 Jul;39(1):21-9.

15.Bohrer MP, Baylis C, Humes HD et al: Permselectivity of the glomerular capillary wall Facilitated filtration of circulating polycations. J Clin Invest 61;72,1978.

16.Brown EA, Upadhyaya K, Hayslett JP, Kashgarian M, Siegel NJ. The clinical course of mesangial proliferative glomerulonephritis: Medicine (Baltimore). 1979 Jul;58(4):295-303.

17.Burkholder PM, Marchand A, Kreuger RP: Mixed Membranous and proliferative Glomerulonephritis: A correlative Light, Immunofluorescence, and Electron Microscopic Study. Lab Invest 1970;23:459-479.

18.Burkholder PM: Glomerulonephritis. International conference on pathogenesis, pathology and treatment: Morphologic basis for classification of glomerulonephritis, Edited by Kluthe R Vogt A, Batsford S, New York, Wiley J and sons 1977; 54-79 (cited in 39)

19.Cameron JS, Turner DR, Ogg CS, Sharpstane P and Brown CB: The nephrotic syndrome in adults with 'minimal change' glomerular lesions. Q J Med NS 1974; 43:461 (cited in 14)

20.Cameron JS, Turner DR. Ogg CS, Chantler C, Williams DG: The long term prognosis of patients with focal segmental glomerulosclerosis: Clin Nephrl.1978 Dec;10(6): 213-8.

21.Castlemen and Smith Renal biopsy - Shanbagh and Joshi J.Assocn of phyn.of Indian Vol. 31, P. No.12-1983.

22.Chandrasekaran Venkataraman Edwin Fernando M, Kandasamy S. Jayakumar M. Spectrum of Renal diseases in Elderly patients: 33-rd Annual Conference of Indian Society of Nephrology CN-P-16-27.-2002.

23.Chaturvedi M, Jain A, Agarwal D, Malhotra V, Pareek SK. Renal biopsy analysis in adult nephrotics in atertiary level hospital:33rd Annual Conference of Indian Society of Nephrology CN-P-16-30 2002.

24. Cheigh JS, Mouradian J, Soliman M, Tapia L, Riggio RR, Stenzel KH, Rubin AL. Focal segmental glomerulosclerosis in renal transplants: Am J Kidney Dis.1983 Jan;2 (4):449-55.

25.Churg J, Habib R, whit RHR, pathology of the syndrome in children: A report for the international study of kidney disease in children. Lancet 1970; 1:1299-1302.

26.Churg J, Grishman E: Application of thin sections to the problems of renal pathology.J Mt Sinai Hosp 24:736,1957.

27.Clarkson AR. Wondroffe AJ. Aarons I: IgA nephropathy and Henoch-Schonlein purpura In Schrier RW, Gottschalk CW, eds: Diseases of the kidney. Ed 5, Vol H. Boston. 1993, Little. Brown & Co. pp. 1839-1864.

28.Coons AH, Kaplan MH: Localisation of antigen in tissue cells.Improvement in a method for the detection of antigen by means of fluorescent antibody.J Exp Med 91:1,1950.

29.Cosslett VE: Practical electron microscopy. Academic Pres, San Diego, 1951. Renal Biopsy interpretation 1996. P No. 2-18.

30.Cruz HM, Penna Dde O, Saldanha LB, Cruz J, LuizP. Mareondes M.(Histopathologic study of primary glomerulopathies: Retrospective analysis of 197 renal biopsies (1985-1987): Rev Hosp Clin Fac Med Sao Paulo. 1989 May- Jun; 44 (3):94-9.

31.Daniel L, Saingra Y, Glorgl R, Bouvier C, Pellissier JF, Berland Y. Tubular lesions determine prognosis of IgA nephropathy: Am J Kidney Dis. 2000 Jan; 35(1):13-20.

32. Danilewiez M, Wagrowska – Danilewiez M.: Idiopathic membranous glomeulonephritis: a quantitative study of glomerular and interstitial lesions: Pol J Pathol . 1995;46(3):173-7.

33. Davis A. Schneeberger E, Grupe W et al: Membranoproliferativeg Glomerulonephritis (MPGN type 1) and dense deposit disease (DDD) in children. C;om Me[jrp; 1978; 95:184 – 193 (cited in 39)

34. Decramer S. (Article in French) (Berger's disease in Childhood): Arch Pediatr. 2002 May:9 (5): 519-29.

35. Dixon FJ, Feldman JD: Experimental giomerulonephritis J Expt Med,1961.

36. Ellis AWM : Natural history of Bight's disease. Clinical, Histological and environmental observations. Lancet 1:1, 34, 72, 1942.

37.Fernandez Moran H: Application of diamond knife for ultrathin sectioning to the study of the fine structure of biological tissue and metals.J Biophys Biochem Cytol,sppl,2:29,1956.

38.Ferrario F, Rastali MP.: Histopathological atlas of renal disease: Membranous glomerulonephritis: J Nephrol, 2004 Sep-Oct;17(5): 635-6.

39. Fox A: Light microscopy of glomerular disease. Minn Med 1975;58: 68-87 (cited in 39)

40. Galla JH. IgA Nephropathy. Kidney Int. 47:377-387, 1995:

41. Garty BZ, Eisenstein B, Sandbank J, Kaffe S. Degan R. Gadoth N: Microcephaly and congenital nephritic syndrome owing to diffuse mesangial sclerosis. An autosomal recessive syndrome in the I-st year of life. Pediatr Nephrol 7:327,353,1993.

42. Genel F, Arslanoglu S, Durmaz B, Bak M. Primary IgA nephropathy in children: association of clinical and pathological findings with prognosis: Indian J Pediatr. 2001 May'68(5):409-12.

43. Glasgow EF,Mancrieff MW and White RHR: Symptomless haematuria in childhood.Br Med J1970;2:687(cited in 14)

44. Glassock RJ: Syndromes of glomerular diseases. P.681. In Massry SG, **Glassock RJ (eds):** Textbook of Nephrology. 3rd Ed. Williams & Wilkins, Baltimore, 1995. glomerular capillary wall. Facilitated filtration of, circulating glomeulopathy in adulthood. Am J Nephrol 8:291, 1988.

45. Gwyn NB: Biopsies and the completion of certain surgical procedures. Can Med Assoc J 13:820, 1923.

46. Habib R. Nephrotic syndrome in the I-st year of life: Paediatric Nephrology 1993 Aug;7 (4):347-53.

47. Haque SF, Khan AS. Clinicopathological correlation inadult- onset Nephrotic syndrome: 33rd Annual Conference of Indian society of Nephrology CN-P-16-61, 2002;

48. Hass M, Spargo BH, Coventry S: Increasing incidence of focal-segmental glomerulosclerosis among adult nephropathies: a 20 year renal biopsy study: Am J Kidney Dis.195 Nov; 26 (5):740-50.

49. Hayslett JP, Kashgarian M, Bensch KG, Spargo BH, Freedman LR and Epstein FH: Clinicopathological correlation in the nephritic syndrome due to primary renal disease, Medicine 1973' 52; 93-120.

50. Herdson PP, Jennings RB and Early DP, Chicago: Glomerular fine stricture in post streptococcal acute glomerulonephritis. Arch path 1966;81:117-128.

51. Hill GS, Hinglais N, Tran F, Bach JF: Systemic Lupus eryuthematosus: Morphologic correlations with immunologic and clinical data at the time of biopsy. Am J Med 1978;64:61-79.

52. Hogg RJ. IgA nephropathy: clinical features and natural history – a pediatric perspective: Am J Kidney Dis. 1988 Nov;12 (5):358-61.

53. Holmberg C. Jalanko H. Koskimies O. Leijala M. Salmela K. Eklund B. Ahonen J: Renal transplantation in small children with congenital nephritic syndrome of the Finnish type. Transplant Proc 23:1378-1379,1991.

54. Howie AJ, Lee SJ, Green NJ, Newbold KM, Kizaki T. Koram A, Richards NT, Micheal J, Adu D. Different clinicopathological types of segmental sclerosing glomerular lesions in adults: Nephrol Dial Transplant.1993;8(7):590-9.

55. Howie AJ. Pathology of minimal change nephropathy and segmental sclerosing glomeular disorders: Nephrol DialTransplant. 2003 Aug;18 Suppl 6:vi33-8.

56. Huong DL, Papo T, Beaufils H, Wechsler B, Bletry O, Baumelou A, Godeau P, Piette JC:. Renal involvement in systemic lupus erythematous. A study of 180 patients from a single center:Medicine (Baltimore). 1999 May;78(3):148-66.

57. Huttunen N-P: Congenital nephritic syndrome of Finnish type. Study of 75 patients. Arch Dis Child 51:344:348.1976.

58. International study of Kidney diseases in Children: Primary Nephrotic syndrome in children: clinical significance of histopathologic variant of minimal change and of diffuse mesangial hypercellularity. Kidney Int. 20:765,1981.

59. Iverson P, Brun C: Aspiration biopsy of the kidney Am J Med 11: 324,1951.

60. Jennett JC, Iskandan SS, Dalldorf FG: Pathologic differentiation between lupus and Non lupus membranous glomerulopathy. Kidney Int 24:377 – 385. 1983.

61. Jennings RB and Earle DP: Post streptococcal glomerulonephritis. Histopathologic and clinical studies of the acute, subsiding acute and early chronic latent phases. J Clin Invest 1961; 40:1525 (cited in 56).

62. Jones DB: Inflammation and repair of the glomerulus. Am J Pathol 27:91,1951.

63. Jones DB: Mucosubstances of the glomerulus,Lab invest 21:119, 1969. Renal Biopsy interpretation 1996. P No. 2-18.

64. Jones DB:Glomerulonephritis. Am J Pathol 29:33, 1953.

65. Kark RM, Muehrcke RC: Biopsy of the kidney in prone position, lancet 1:1047, 1954. Renal Biopsy interpretation 1996. P No. 2-18.

66. Kaysen GA, Schonanfeld Y: Albumin homeostasis in Patients Undergoing continuous ambulatory peritoneal dialysis. Kidney Int. 25:107,1984

67. Korbet SM, Schwartz mm., Lewis EJ:. Minimal-change glomerulopathy of adulthood: Am J Nephrol. 1988;8(4):291-7.

68. Koziell A,Iyer VK,Moghul NE, Ramani P, Taylor CM: Congenital nephritic syndrome . Pediatr Nephrol, 2001Feb: 16(2):190-1.

69. Krakower CA, Greenspan SA: Localisation of the nephrotoxic antigen within the isolated renal glomerulus.Arch Pathol 51:629,1951.

70.Kumar J, Gulati S, Sharma AP, Sharma RK, Gupta RK. Histopathological spectron of child hood Nephrotic syndrome in Indian children: Pediatr Nephrol-2003 Jul;18(7): 657- 60, Epub 2003 May 13.

71.Kuroki A, Shibata T, Honda H, Totsuka D, Kobayashi K, Sugisaki.T. Glomerular and serum IgG subclasses in diffuse proliferative lupus nephritis, membranous lupus nephritis, and diopathic membranous nephropathy: Intern Med. 2002 Nov;41 (11):936-42.

72. Lawrence JR, Pollak VE, Pirani CL and Kark RM: Histologic and clinical evidence of post streptococcal glomerulonephritis in patients with the nephritic syndrome. Medicine 1963; 42:1 (cited in 17).

73. Le J, Vilcek J: Interleukin 6: a multifunctional cytokine regulating immune reactions and the acute phase protein response. Lab Invest 61:588, 1989. Renal Biopsy interpretation 1996. P No. 2-18.

74. Lindblom K: Percutaneous puncture of renal cysts and tumors. Acta Radiol 27: 66, 1946.

75. Mackay IR and Taft LT: Renal biopsy with particular reference to the study of diabetes mellitus, systemic lupus erythematosus, and subacute glomerulonephritis. Aust ann Med 1961; 10:178 (cited in 17)

76. Markowitz GS, Schwimmer JA, Stokes NMB, Nasr S, Seigle RL, Valeri Am, D' Agati VD. C1q nephropathy: a variant of focal segmental glomerulosclerosis: Kidney Int. 2003 Oct;64(4):1232.

77. Mc Manus JFK : The peiodic acid routine applied to the kidney. Am J Pathol 24:643,1948.

78. Mazzarolo Cruz HM, Cruz J, Silva AL Jr. Saldanha LB, de Oliveira Penna D. Prevalence of adult primary glomerular diseases: Retrospective analysis of 206 kidney biopsies (1990-1993): Rev. Hosp Clin Fac Med Sao Paulo. 1996 Jan- Feb; 51 (1): 3-6.

79. Noel LH, Zanetti M. Drkoz. D. Barbanel C: Long term prognosis of Idiopathic Membranous Glomerulonephrosis: Study of 116 untreated patients. AM J Med 1979; 66; 82-90

80. Passos EM, Legallicier B, Godin M.: Membranous Nephropathy: Rev Prat, 2003 Nov 30;53 (18):2033-8.

81. Polenakovic M, Grcevska L:. (Disordes of renal function in patients with minimal change glomerulopathy): Srp Arh Celok Lek. 1996;124 Suppl 1:55-7.

82. Postishil' IuA.: (Pathomiorphology of kidney lesions induced by non-steroidal anti-inflammatory drugs): Arkh Patol. 1995 Sep-Oct;57(5): 70-4.

83.Report of the International study of Kidney Disease in Children. Nephrotic syndrome in children. Prediction of histopathology from clinical and laboratory characteristics at time of diagnosis. Kidney Int. 13:159-165. 1978.

84. Sabatini DD, Bensch KG, Barnett RJ: Cytochemistry and electron microscopy. The preservation of cellular ultrastructure andenzymatic activity by aldehyde fixation. J Cell Biol 17:19,1963.

85. Sasagawa Y. A clinico-pathological study on progress factors in lupus nephritis: Nippon Jinzo Gakkai Shi. 1993 Apr;35(4):399-410.

86. Seppala M. Rapiola J, Huttunen NP, Aula P, Karjalainen O, Ruoslahti E: Congenital nephrotic syndrome: prenatal diagnosis and genetic counseling by estimation of aminotic – fluid and maternal serum alpha-fetoprotein: Lancet, 1976 Jul 17;2 (7977):123-5.

87. Shiki H, Dohi K, Primary focal segmental glomerulo sclerosis: Clnical course, Predictors of Renal outcome and treatement. Intern Med. 2000 Aug;39(8):606-11.

88. Sibley RK. Striegel J: Nephrotic syndrome in the first year of life. In Tisher CC. Brenner BM. Eds: Renal pathology with clinical and functional correlations, ed 2, Vol I. Philadelphia. 1994, JB Lippincott Co. pp 1291-1311.

89. Srivastava T, Simon SD, Alon US. High incidence of ocal segmental glomerulosclerosis in nephrotic syndrome of childhood: Pediatr Nephrol. 1999 Jan;13 (1):13-8.

90. Tang Z, Wu Y, Wang QW, yu, Hu Wx, YAO xd,Chen HP,Liu ZH, Li LS. Idiopathic IgA Nephropathy with diffuse crescent formation.:Am J Nephrol. 2002 Sep- Dec; 22(5-6):400-6.

91. Toth T, Takebayashi S.: Idiopathic membranous glomeruloephritis: a clinicopthologic and quantitative morphometric study: Clin.Nephrology 1992 Jul;38(1):14-9.

92. Vande Walle JG, Donckerwolcke RA. Pathogenesis of oedema formation in nephritic syndrome: Pediatr. Nephrol.2000 Mar: 16(3):283-93.

93. Volhard F, Fahr, T: Die Brightische Nierenkrankheit, springer, Berlin 1914. Renal Biopsy interpretation 1996. P No. 2-18.

94. Von-pirquet and Smith Allergy Arch.Of Int MED.7:259-383,1911.

95. Wang S, Kim JH, Moon KC, HJong HK, Lee HS. Cell – Cycle mechanisms involved in podocyte proliferation in cellular lesion of focal segmental glomerulosclerosis: Am J Kidney Dis. 2004 Jan; 43(1):19-27.

96. White RHR, Glasscow EF, Mills RJ: Clinicopathiologic study of nephrotic syndrome in childhood. Lancet 1970; 1: 1353-1359.

97. Yoshioka T, Rennke HG, Salant DJ et al: Role of abnormally high transmural pressure in the permselectivity defect of glomerular capillary wall: a study in early passive Heymann nephritis. Cire Res 61:531,1987.